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(54) Title: NOVEL UBIQUITIN LIGASES AS THERAPEUTIC TARGETS
(54) Titre: NOUVELLES UBIQUITINE LIGASES UTILES COMME CIBLES THERAPEUTIQUES

(57) Abstract

The present invention relates to the discovery, identification and characterization of nucleotides that encode novel substrate-targeting subunits of ubiquitin ligases. The invention encompasses nucleotides encoding novel substrate-targeting subunits of ubiquitin ligases: FBP1, FBP2, FBP3, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, transgenic mice, knock-out mice, host cell expression systems and proteins encoded by the nucleotides of the present invention. The present invention relates to screening assays that use the novel substrate-targeting subunits to identify potential therapeutic agents such as small molecules, compounds or derivatives and analogues of the novel ubiquitin ligases which modulate activity of the novel ubiquitin ligases for the treatment of proliferative and differentiative disorders, such as cancer, major opportunistic infections, immune disorders, certain cardiovascular diseases, and inflammatory disorders. The invention further encompasses therapeutic protocols and pharmaceutical compositions designed to target ubiquitin ligases and their substrates for the treatment of proliferative disorders.

(57) Abrégé

La présente invention concerne la découverte, l'identification et la caractérisation de nucléotides codant pour de nouvelles sous-unités d'ubiquitine ligases ciblant un substrat. L'invention concerne des nucléotides codant pour de nouvelles sous-unités d'ubiquitine ligases ciblant un substrat: FBP1, FBP2, FBP3, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24 et FBP25, des souris transgéniques, des souris _ knock-out _, des systèmes d'expression cellulaires hôtes et des protéines codées par les nucléotides de l'invention. L'invention a trait à des techniques de criblage utilisant les nouvelles sous-unités ciblant un substrat pour identifier des agents thérapeutiques potentiels tels que de petites molécules, des composés ou dérivés, et des analogues des nouvelles ubiquitine ligases qui modulent l'activité des nouvelles ubiquitine ligases, en vue de traiter des troubles de prolifération et de différenciation cellulaires tels que le cancer, des infections opportunistes majeures, des troubles immunitaires, certaines maladies cardio-vasculaires et des maladies inflammatoires. L'invention concerne de plus des protocoles thérapeutiques et des compositions pharmaceutiques conçues pour cibler des ubiquitine ligases et leurs substrats, en vue du traitement de troubles de prolifération cellulaire.

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(54) Title: NOVEL UBIQUITIN LIGASES AS THERAPEUTIC TARGETS

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The present invention relates to the discovery, identification and characterization of nucleotides that encode novel substrate–targeting subunits of ubiquitin ligases. The invention encompasses nucleotides encoding novel substrate–targeting subunits of ubiquitin ligases: FBP1, FBP2, FBP3, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, transgenic mice, knock–out mice, host cell expression systems and proteins encoded by the nucleotides of the present invention. The present invention relates to screening assays that use the novel substrate–targeting subunits to identify potential therapeutic agents such as small molecules, compounds or derivatives and analogues of the novel ubiquitin ligases which modulate activity of the novel ubiquitin ligases for the treatment of proliferative and differentiative disorders, such as cancer, major opportunistic infections, immune disorders, certain cardiovascular diseases, and inflammatory disorders. The invention further encompasses therapeutic protocols and pharmaceutical compositions designed to target ubiquitin ligases and their substrates for the treatment of proliferative disorders.

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Description

NOVEL UBIQUITIN LIGASES AS THERAPEUTIC TARGETS

1. INTRODUCTION

The present invention relates to the discovery, identification and characterization of nucleotide sequences that encode novel substrate-targeting subunits of ubiquitin ligases. The invention encompasses nucleic acid molecules comprising nucleotide sequences encoding novel substrate-targeting subunits of ubiquitin ligases: FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP11, FBP12, FBP13, FBP14, FBP15, FBP17, FBP18, FBP20, FBP21, FBP22, FBP23, AND FBP25, transgenic mice, knock-out mice, host cell expression systems and proteins encoded by the nucleotides of the present invention. The present invention relates to screening assays to identify potential therapeutic agents such as small molecules, compounds or derivatives and analogues of the novel ubiquitin ligases which modulate activity of the novel ubiquitin ligases for the treatment of proliferative and differentiative disorders, such as cancer, major opportunistic infections, immune disorders, certain cardiovascular diseases, and inflammatory disorders. The invention further encompasses therapeutic protocols and pharmaceutical compositions designed to target ubiquitin ligases and their substrates for the treatment of proliferative disorders.

2. BACKGROUND OF THE INVENTION

2.1 CELL CYCLE REGULATORY PROTEINS

The eukaryotic cell cycle is regulated by a family of serine/threonine protein kinases called cyclin dependent kinases (Cdks) because their activity requires the association with regulatory subunits named Cyclins (Hunter & Pines, 1994, Cell 79:573). Cdks also associate with Cdk inhibitors (Ckis) which mediate cell cycle arrest in response to various antiproliferative signals. So far, based on their sequence homology, two families of Ckis have been identified in mammalian cells: the Cip/Kip family, which includes p21, p27 and p57; and the Ink family, which includes p15, p16, p18, and p20 (Sherr & Roberts, 1999, Genes & Dev. 13: 1501).

2.2 THE UBIQUITIN PATHWAY

Ubiquitin-mediated proteolysis is an important pathway of non-lysosomal protein degradation which controls the timed destruction of many cellular regulatory proteins including, p27, p53, p300, cyclins, E2F, STAT-1, c-Myc, c-Jun, EGF receptor,

IkBα, NFkB and β-catenin (reviewed in Pagano, 1997, FASEB J. 11: 1067). Ubiquitin is an evolutionary highly conserved 76-amino acid polypeptide which is abundantly present in all eukaryotic cells. The ubiquitin pathway leads to the covalent attachment of a poly-ubiquitin chain to target substrates which are then degraded by the multi-catalytic proteasome complex (see Pagano, supra, for a recent review). Many of the steps regulating protein ubiquitination are known. Initially the ubiquitin activating enzyme (E1), forms a high energy thioester with ubiquitin which is, in turn, transferred to a reactive cysteine residue of one of many ubiquitin conjugating enzymes (Ubcs or E2s). The final transfer of ubiquitin to an e-amino group of a reactive lysine residue in the target protein occurs in a reaction that may or may not require an ubiquitin ligase (E3) protein. The large number of ubiquitin ligases ensures the high level of substrate specificity.

2.3 THE UBIQUITIN PATHWAY AND THE REGULATION OF THE G1 PHASE BY F BOX PROTEINS

Genetic and biochemical studies in several organisms have shown that the G1 phase of the cell cycle is regulated by the ubiquitin pathway. Proteolysis of cyclins, Ckis and other G1 regulatory proteins is controlled in yeast by the ubiquitin conjugating enzyme Ubc3 (also called Cdc34) and by an E3 ubiquitin ligase formed by three subunits: Cdc53, Skp1 and one of many F box proteins (reviewed in E. Patton et al., 1998, TIG. 14:6). The F box proteins (FBPs) are so called because they contain a motif, the F box, that was first identified in Cyclin F, and that is necessary for FBP interaction with Skp1 (Bai, et al., 1996, Cell 86:263). In addition, F box proteins also contain either WD-40 domains or Leucine-Rich Repeats (LRR) protein-protein interaction domains. Cdc53 (also called Cul A) and Skp1 appear to participate in the formation of at least three distinct E3, each containing a different F box protein. Because these ligases are similar protein modules composed of Skp1, Cul A, and an F box protein, they have been named SCF. The interaction of the ligase with its substrates occurs via the F box subunit. The three SCFs identified so far in S. cerevisiae are: SCF^{Cdc4} (which recruits the Ckis Sic1 and Far1, the replication factor Cde6, and the transcriptional activator Gcn4, as substrates through the F box protein Cdc4), SCF^{Grr1} (which recruits the G1 cyclins Cln1 and Cln2 as substrates through the F box protein GRR1), and SCF^{Met30} (which recruits the G1 cyclin Cln3 as a substrate throughout the F box protein MET30; see Pagano and Patton, supra, for recent reviews).

The intracellular level of the human Cki p27 is mainly regulated by degradation and it is known that the ubiquitin system controls p27 degradation (Pagano et

al., 1995, Science 269:682). Similarly, degradation of other G1 human regulatory proteins (Cyclin E, Cyclin D1, p21, E2F, β-catenin) is controlled by the ubiquitin-pathway (reviewed in M. Pagano, supra). Yet, the specific enzymes involved in the degradation of G1 regulatory proteins have not been identified.

A family of 6 genes (*CUL1, 2, 3, 4a, 4b,* and *5*) homologous to S. cerevisiae cul A have been identified by searching the EST database (Kipreos, et al., 1996, Cell 85:829). Human Skp1 and the F box protein Skp2 (that contains five LRRs) were identified as two proteins associated in vivo with Cyclin A and thus designated as S-phase kinase-associated protein 1 and 2 (Zhang, et al., 1995, Cell 82:915).

2.4 DEREGULATION OF THE UBIQUITIN PATHWAY IN CANCER AND OTHER PROLIFERATIVE DISORDERS

Cancer develops when cells multiply too quickly. Cell proliferation is determined by the net balance of positive and negative signals. When positive signals overcome or when negative signals are absent, the cells multiply too quickly and cancer develops.

Ordinarily cells precisely control the amount of any given protein and eliminate the excess or any unwanted protein. To do so, the cell specifically tags the undesired protein with a long chain of molecules called ubiquitin. These molecules are then recognized and destroyed by a complex named proteasome. However, all this mechanism goes awry in tumors leading to the excessive accumulation of positive signals (oncogenic proteins), or resulting in the abnormal degradation of negative regulators (tumor suppressor proteins). Thus, without tumor suppressor proteins or in the presence of too much oncogenic proteins, cells multiply ceaselessly, forming tumors (reviewed by Ciechanover, 1998, EMBO J. 17: 7151; Spataro, 1998, Br. J. Cancer 77: 448). For example, abnormal ubiquitin-mediated degradation of the p53 tumor suppressor (reviewed by J. Brown and M. Pagano, 1997, Biochim. Biophys. Acta1332: 1), the putative oncogene β-catenin (reviewed by Peifer, 1997, Science 275:1752) and the Cki p27 (reviewed in Ciechanover, supra; Spataro, supra, Lloyd, 1999, Am. J. Pathol.154: 313) have been correlated with tumorigenesis, opening to the hypothesis that some genes encoding ubiquitinating enzymes may be mutated in tumors.

Initial evidence indicates that human F-box proteins play a role in the ubiquitination of G1 regulatory proteins as their homologs do in yeast (see below). Unchecked degradation of cell cycle regulatory proteins has been observed in certain tumors and it is possible that deregulated ubiquitin ligase play a role in the altered degradation of cell cycle regulators. A well understood example is that of Mdm2, a

ubiquitin ligase whose overexpression induces low levels of its substrate, the tumor suppressor p53.

3. SUMMARY OF THE INVENTION

The present invention relates to novel F box proteins and therapeutic protocols and pharmaceutical compositions designed to target the novel F box proteins and their interactions with substrates for the treatment of proliferative and differentiative disorders. The present invention also relates to screening assays to identify substrates of the novel F box proteins and to identify agents which modulate or target the novel ubiquitin ligases and interactions with their substrates. The invention further relates to screening assays based on the identification of novel substrates of known F box proteins, such as the two novel substrates of the known F box protein Skp2, E2F and p27. The screening assays of the present invention may be used to identify potential therapeutic agents for the treatment of proliferative or differentiative disorders and other disorders that related to levels of expression or enzymatic activity of F box proteins.

The invention is based in part, on the Applicants' discovery, identification and characterization of nucleic acids comprising nucleototide sequences that encode novel ubiquitin ligases with F box motifs. These twenty-six novel substrate-targeting subunits of ubiquitin ligase complexes, FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, described herein, were first identified based on their interaction with components of the ubiquitin ligase complex (FBP1, FBP2, FBP3a, FBP4, FBP5, FBP6 and FBP7) or by sequence comparison of these proteins with nucleotide sequences present in DNA databases (FBP3b, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25). These novel substrate-targeting subunits of ubiquitin ligase complexes each contain an F box motif through which they interact with the other components of the ubiquitin ligase complex. In addition, some of these FBPs contain WD-40 domains and LRRs (which appear to be involved in their interaction with substrates), while other FBPs contain potential protein-protein interaction modules not yet identified in FBPs, such as leucine zippers, ring fingers, helix-loop-helix motifs, proline rich motifs and SH2 domains. The invention is also based, in part, on the Applicants' discovery and identification of FBP specific substrates p27 and β-catenin and on methods to identify novel FBP substrates. Some of the genes encoding the novel F box proteins were also mapped to chromosome sites frequently altered in breast, prostate and ovarian cancer,

nasopharyngeal and small cell lung carcinomas, gastric hepatocarcinomas, Burkitt's lymphoma and parathyroid adenomas. Finally, the invention is also based, in part, on the Applicants' generation of transgenic mice expressing wild type or dominant negative versions of FBP proteins and on the generation of FBP knock-out mice.

The invention encompasses the following nucleotide sequences, host cells expressing such nucleotide sequences, and the expression products of such nucleotide sequences: (a) nucleotide sequences that encode mammalian FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP11, FBP12, FBP13, FBP14, FBP15, FBP17, FBP18, FBP20, FBP21, FBP22, FBP23, and FBP25, including the human nucleotides, and their gene products; (b) nucleotides that encode portions of the novel substrate-targeting subunits of ubiquitin ligase complexes, and the polypeptide products specified by such nucleotide sequences, including but not limited to F box motifs, the substrate binding domains; WD-40 domains; and leucine rich repeats, *etc.*; (c) nucleotides that encode mutants of the novel ubiquitin ligases in which all or part of the domain is deleted or altered, and the polypeptide products specified by such nucleotide sequences; (d) nucleotides that encode fusion proteins containing the novel ubiquitin ligases or one of its domains fused to another polypeptide.

The invention further encompasses agonists and antagonists of the novel substrate-targeting subunits of ubiquitin ligase complexes, including small molecules, large molecules, mutants that compete with native F box binding proteins, and antibodies as well as nucleotide sequences that can be used to inhibit ubiquitin ligase gene expression (*e.g.*, antisense and ribozyme molecules, and gene regulatory or replacement constructs) or to enhance ubiquitin ligase gene expression (*e.g.*, expression constructs that place the ubiquitin ligase gene under the control of a strong promoter system), and transgenic animals that express a ubiquitin ligase transgene or knock-outs that do not express the novel ubiquitin ligases.

Further, the present invention also relates to methods for the use of the genes and/or gene products of novel substrate-targeting subunits of ubiquitin ligase complexes for the identification of compounds which modulate, *i.e.*, act as agonists or antagonists, of ubiquitin ligase activity. Such compounds can be used as agents to control proliferative or differentiative disorders, *e.g.* cancer. In particular, the present invention encompasses methods to inhibit the interaction between β-catenin and FBP1 or p27 and Skp2. In fact, agents able to block these interactions can be used to modulate cell proliferation and/or growth.

Still further, the invention encompasses screening methods to identify derivatives and analogues of the novel substrate-targeting subunits of ubiquitin ligase

complexes which modulate the activity of the novel ligases as potential therapeutics for proliferative or differentiative disorders. The invention provides methods of screening for proteins that interact with novel components of the ubiquitin ligase complex, including FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 or derivatives, fragments or domains thereof, such as the F box motif. In accordance with the invention, the screening methods may utilize known assays to identify protein-protein interactions including phage display assays or the yeast two-hybrid assay system or variations thereof.

In addition, the present invention is directed to methods that utilize FBP gene sequences and/or FBP gene product sequences for the diagnostic evaluation, genetic testing and/or prognosis of an FBP-related disorder, such as a proliferative disorder. For example, the invention relates to methods for diagnosing FBP-related disorders, *e.g.*, proliferative disorders, wherein such methods can comprise measuring FBP gene expression in a patient sample, or detecting an FBP mutation that correlates with the presence or development of such a disorder, in the genome of a mammal suspected of exhibiting such a disorder. In particular, the invention encompasses methods for determining if a subject (*e.g.*, a human patient) is a risk for a disorder characterized by one or more of: (i) a mutation of an FBP gene encoding a protein represented in part A of Figures 3-28, or a homolog thereof; (ii) the mis-expression of an FBP gene; (iii) the mis-expression of an FBP protein.

The invention is illustrated by way of working examples which demonstrate the identification and characterization of the novel substrate-targeting subunits of ubiquitin ligase complexes. The working examples of the present invention further demonstrate the identification of the specific interaction of (i) FBP1 with β-catenin and (ii) the known FBP, Skp2, with the cell-cycle regulatory proteins E2F and p27. These interactions suggest that β-catenin is a specific substrate of FBP1, while E2F and p27 are substrates of Skp2. In fact, the working examples of the present invention further demonstrate that β-catenin is a specific substrate of FBP1, while p27 is substrates of Skp2. The identification of proteins interacting with the novel FBPs will be possible using the methods described herein or with a different approach.

3.1 DEFINITIONS

As used herein, the term "F-box motif" refers to a stretch of approximately 40 amino acid that was identified as being necessary for the interaction of F-box containing

proteins with Skp1. The consensus sequence of an F-box motif is described in Bai et al., 1996, Cell 86:263-274, incorporated herein by reference in its entirety.

As used herein the term "F-box protein" (FBP) refers to peptide, polypeptide or protein which contains an F-box motif.

Although, FBPs are substrate-targeting subunits of ubiquitin ligase complexes, as used herein the term "ubiquitin ligase" refers to a peptide, polypeptide or protein that contains an F-box motif and interacts with Skp1.

As used herein, the term "functionally equivalent to an FBP gene product" refers to a gene product that exhibits at least one of the biological activities of the endogenous FBP gene product. For example, a functionally equivalent FBP gene product is one that is capable of interacting with Skp1 so as to become associated with a ubiquitin ligase complex. Such a ubiquitin ligase complex may be capable of ubiquitinating a specific cell-cycle regulatory protein, such as a cyclin or cki protein.

As used herein, the term "to target" means to inhibit, block or prevent gene expression, enzymatic activity, or interaction with other cellular factors.

As used herein, the term "therapeutic agent" refers to any molecule, compound or treatment that alleviates of assists in the treatment of a proliferative disorder or related disorder.

As used herein, the terms "WD-40 domain", "Leucine Rich Repeat", "Leucine Zipper", "Ring finger", "Helix-loop-helix motif", "Proline rich motif", and "SH2 domain" refer to domains potentially involved in mediating protein-protein interactions. The "WD-40 domain" refers to a consensus sequence of forty amino acid repeats which is rich in tryptophan and aspartic acid residues and is commonly found in the beta subunits of trimeric G proteins (see Neer et al., 1994 Nature 371:297-300 and references therein, which are incorporated herein by reference in their entirety). An "LRR" or a "Leucine Rich Repeat" is a leucine rich sequence also known to be involved in mediating protein-protein interactions (see Kobe & Deisenhofer, 1994, Trends. Biochem. Sci. 19:415-421 which are incorporated herein by reference in their entirety). A "leucine zipper" domain refers to a domain comprising a stretch of amino acids with a leucine residue in every seventh position which is present in a large family of transcription factors (see Landshultz et al., 1988, Science 240:1759-64; see also Sudol et al., 1996, Trends Biochem. 21:1-3, and Koch et al., 1991, Science 252:668-74).

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Alignment of the conserved F-box motif amino acid residues in the human F-box proteins FBP1 (SEQ ID NO:15) , FBP2 (SEQ ID NO:16), FBP3a (SEQ ID NO:17), FBP3b (SEQ ID NO:78), FBP4 (SEQ ID NO:18), FBP5 (SEQ ID NO:19), FBP6 (SEQ ID NO:20), FBP7 (SEQ ID NO:21), Skp2 (SEQ ID NO:22), FBP8 (SEQ ID NO:61) FBP9 (SEQ ID NO:62), FBP10 (SEQ ID NO:63), FBP11 (SEQ ID NO:64), FBP12 (SEQ ID NO:65), FBP13 (SEQ ID NO:79); FBP14 (SEQ ID NO:66); FBP15 (SEQ ID NO:67), FBP16 (SEQ ID NO:68), FBP17 (SEQ ID NO:69), FBP18 (SEQ ID NO:70), FBP19 (SEQ ID NO:71), FBP20 (SEQ ID NO:72), FBP21 (SEQ ID NO:73), FBP22 (SEQ ID NO:74), FBP23 (SEQ ID NO:75), FBP24 (SEQ ID NO:76), FBP25 (SEQ ID NO:77). Alignment of the F-boxes of a previously known FBP, Skp2, with the F-boxes of FBPs identified through a two-hybrid screen (designated by the pound symbol) or BLAST searches (designated by a cross) was performed using the Clustal W method (MacVector(tm)) followed by manual re-adjustment. Identical residues in at least 15 F-boxes are shaded in dark gray, while similar residues are shaded in light gray. One asterisk indicates the presence in the cDNA of a STOP codon followed by a polyA tail, while potential full length clones are designated with two asterisks. The asterisks on the bottom of the figure indicate the amino acid residues mutated in FBP3a (see Figure 29).

FIG. 2. Schematic representation of FBPs. Putative protein-protein interaction domains in human FBPs are represented (see key-box for explanation). FBPs identified by a two-hybrid screen are designated by the pound symbol, FBPs identified through BLAST searches by a cross. The double slash indicates that the corresponding cDNAs are incomplete at the 5' end; the asterisks indicate the presence in the cDNA of a STOP codon followed by a polyA tail.

FIG. 3 A-B. **A.** Amino acid sequence of human F-box protein FBP1 (SEQ ID NO:2). **B.** Corresponding cDNA (SEQ ID NO:1).

FIG. 4 A-B. **A.** Amino acid sequence of human F-box protein FBP2 (SEQ ID NO:4). **B.** Corresponding cDNA (SEQ ID NO:3).

FIG. 5 A-B. **A.** Amino acid sequence of human F-box protein FBP3a (SEQ ID NO:6). **B.** Corresponding cDNA (SEQ ID NO:5).

FIG. 6 A-B. **A.** Amino acid sequence of human F-box protein FBP3b (SEQ ID NO:24). **B.** Corresponding cDNA (SEQ ID NO:23).

FIG. 7 A-B. **A.** Amino acid sequence of human F-box protein FBP4 (SEQ ID NO:8). **B.** Corresponding cDNA (SEQ ID NO:7).

FIG. 8 A-B. **A.** Amino acid sequence of human F-box protein FBP5 (SEQ ID NO:10). **B.** Corresponding cDNA (SEQ ID NO:9).

FIG. 9 A-B. **A.** Amino acid sequence of human F-box protein FBP6 (SEQ ID NO:12). **B.** Corresponding cDNA (SEQ ID NO:11).

FIG. 10 A-B. **A.** Amino acid sequence of human F-box protein FBP7 (SEQ ID NO:14). **B.** Corresponding cDNA (SEQ ID NO:13).

FIG. 11 A-B. **A.** Amino acid sequence of human F-box protein FBP8 (SEQ ID NO:26). **B.** Corresponding cDNA (SEQ ID NO:25).

FIG. 12 A-B. **A.** Amino acid sequence of human F-box protein FBP9 (SEQ ID NO:28). **B.** Corresponding cDNA (SEQ ID NO:27).

FIG. 13 A-B. **A.** Amino acid sequence of human F-box protein FBP10 (SEQ ID NO:30). **B.** Corresponding cDNA (SEQ ID NO:29).

FIG. 14 A-B. **A.** Amino acid sequence of human F-box protein FBP11 (SEQ ID NO:32). **B.** Corresponding cDNA (SEQ ID NO:31).

FIG. 15 A-B. **A.** Amino acid sequence of human F-box protein FBP12 (SEQ ID NO:34). **B.** Corresponding cDNA (SEQ ID NO:33).

FIG. 16 A-B. **A.** Amino acid sequence of human F-box protein FBP13 (SEQ ID NO:36). **B.** Corresponding cDNA (SEQ ID NO:35).

FIG. 17 A-B. **A.** Amino acid sequence of human F-box protein FBP14 (SEQ ID NO:38). **B.** Corresponding cDNA (SEQ ID NO:37).

FIG. 18 A-B. **A.** Amino acid sequence of human F-box protein FBP15 (SEQ ID NO:40). **B.** Corresponding cDNA (SEQ ID NO:39).

FIG. 19 A-B. **A.** Amino acid sequence of human F-box protein FBP16 (SEQ ID NO:42). **B.** Corresponding cDNA (SEQ ID NO:41).

FIG. 20 A-B. **A.** Amino acid sequence of human F-box protein FBP17 (SEQ ID NO:44). **B.** Corresponding cDNA (SEQ ID NO:43).

FIG. 21 A-B. **A.** Amino acid sequence of human F-box protein FBP18 (SEQ ID NO:46). **B.** Corresponding cDNA (SEQ ID NO:45).

FIG. 22 A-B. **A.** Amino acid sequence of human F-box protein FBP19 (SEQ ID NO:48). **B.** Corresponding cDNA (SEQ ID NO:47).

FIG. 23 A-B. **A.** Amino acid sequence of human F-box protein FBP20 (SEQ ID NO:50). **B.** Corresponding cDNA (SEQ ID NO:49).

FIG. 24 A-B. **A.** Amino acid sequence of human F-box protein FBP21 (SEQ ID NO:52). **B.** Corresponding cDNA (SEQ ID NO:51).

FIG. 25 A-B. **A.** Amino acid sequence of human F-box protein FBP22 (SEQ ID NO:54). **B.** Corresponding cDNA (SEQ ID NO:53).

FIG. 26 A-B. **A.** Amino acid sequence of human F-box protein FBP23 (SEQ ID NO:56). **B.** Corresponding cDNA (SEQ ID NO:55).

FIG. 27 A-B. **A.** Amino acid sequence of human F-box protein FBP24 (SEQ ID NO:58). **B.** Corresponding cDNA (SEQ ID NO:57).

FIG. 28A-B. **A.** Amino acid sequence of human F-box protein FBP25 (SEQ ID NO:60). **B.** Corresponding cDNA (SEQ ID NO:59).

FIG. 29. FBPs interact specifically with Skp1 through their F-box. The cDNAs of FBPs (wild type and mutants) were transcribed and translated in vitro (IVT) in the presence of 35S- methionine. Similar amounts of IVT proteins (indicated at the top of

each lane) were subjected to a histidine-tagged pull-down assay using Nickel-agarose beads to which either His-tagged-Skp1 (lanes 1, 3, 4, 6-10, 12, 15, 17, 19 and 21), His-tagged-Elongin C (lanes 2, 5, 11, 14, 16, 18, 19 and 22), or His-tagged p27 (lane 12) were pre-bound. Bound IVT proteins were analyzed by SDS-PAGE and autoradiography. The arrows on the left side of the panels point to the indicated FBPs. The apparent molecular weights of the protein standards are indicated on the right side of the panels.

FIG. 30. FBP1, FBP2, FBP3a, FBP4 and FBP7 form novel SCFs with endogenous Skp1 and Cul1 in vivo. HeLa cells were transfected with mammalian expression plasmids encoding Flag-tagged versions of FBP1 (lane 1), (ΔF)FBP1 (lane 2), FBP4 (lane 3), FBP7 (lane 5), FBP2 (lane 7), (ΔF)FBP2 (lane 8), FBP3a (lane 9), (ΔF)FBP3a (lane 10), or with an empty vector (lanes 4 and 6). Cells were lysed and extracts were subjected to immunoprecipitation with a rabbit anti-Flag antibody (lanes 1-8). Immunoprecipitates were then immunoblotted with a mouse anti-Cul1 monoclonal antibody, a rabbit anti-Skp1 polyclonal antibody or a rabbit anti-Cul2 polyclonal antibody, as indicated. The last lane contains 25 µg of extracts from non-transfected HeLa cells; lane 9 contains recombinant Cul1, Skp1, or Cul2 proteins used as markers. The slower migrating bands detected with the antibodies to Cul1 and Cul2 are likely generated by the covalent attachment of a ubiquitin-like molecule to these two cullins, as already described for the yeast cullin Cdc53 and mammalian Cul4a.

FIG. 31. FBP1, FBP2, FBP3a, FBP4 and FBP7 associate with a ubiquitin ligase activity. HeLa cells were transfected with mammalian expression plasmids encoding human Skp1, Cul1 and Flag-tagged versions of FBP1 (lane 3), (ΔF)FBP1 (lane 4), FBP2 (lanes 2 and 5), (ΔF)FBP2 (lane 6), FBP7 (lane 7), FBP3a (lanes 8 and 13), (ΔF)FBP3a (lane 9), a non relevant Flag-tagged protein (Irf3, lane 10), FBP4 (lanes 11 and 12) or with an empty vector (lane 1). Cells were lysed and extracts were subjected to immunoprecipitation with a rabbit anti-Flag antibody. Immunoprecipitates were incubated in the presence of purified recombinant E1 and Ubc4 (lanes 1-11) or Ubc2 (lanes (12 and 13) and a reaction mix containing biotynilated ubiquitin. Reaction in lane 2 contained also NEM. Ubiquitinated proteins were visualized by blotting with HRP-streptavidin. The bracket on the left side of the panels marks a smear of ubiquitinated proteins produced in the reaction, the asterisk indicates ubiquitin conjugated with E1 that were resistant to boiling.

FIG. 32. Subcellular localization of FBPs. HeLa cells were transfected with mammalian expression plasmids encoding Flag-tagged versions of FBP1 (a-b), FBP2 (c-d), FBP3a (e-f), FBP4 (g-h), (DF)FBP2 (i-j), or (ΔF)FBP3a (k-l). After 24 hours, cells were subjected to immunofluorescence with a rabbit anti-Flag antibody (a, c, e, g, i, k) to stain FBPs and bisbenzimide (b, d, f, h, j, l) to stain nuclei.

FIG. 33. Abundance of FBP transcripts in human tissues. Membranes containing electrophoretically fractionated poly(A)+ mRNA from different human tissues were hybridized with specific probes prepared form FBP1, FBP2, FBP3a, FBP4, SKP2, and β-ACTIN cDNAs. The arrows on the left side of the figure point to the major transcripts as described in the text.

FIG. 34A-E. FISH localization of FBP genes. Purified phage DNA containing a genomic probe was labeled with digoxygenin dUTP and detected with Cy3-conjugated antibodies. The signals corresponding to the locus of the genomic probe (red) are seen against the DAPI-Actimomycin D stained normal human chromosomes (blue-white). Panel **A** shows localization of FBP1 to 10q24, **B** shows localization of FBP2 to 9q34, **C** shows localization of FBP3a to 13q22, **D** shows localization of FBP4 to 5p12, and **E** shows localization of FBP5 to 6q25-26. Arrows point to FBP-specific FISH signals.

FIG. 35A-C. FBP1 associates with β-catenin. **A.** Extracts from baculovirus-infected insect cells expressing either β-catenin alone (lane 1) or in combination with Flag-tagged FBP1 (lane 2) were immunoprecipitated (IP) with a rabbit anti-Flag antibody (rα-Flag), followed by immunoblotting with anti-Flag (mα-Flag) and anti-β-catenin mouse antibodies, as indicated. Lanes 3 and 4 contain 25 μg of extracts from infected insect cells immunoblotted with the same antibodies. **B.** Extracts from baculovirus-infected insect cells expressing cyclin D1, Flag-FBP1 in the absence (lanes 1-3) or in the presence of Skp1 (lanes 4-6) were immunoprecipitated with normal rabbit IgG (r-IgG, lanes 1 and 4), rabbit anti-Flag antibody (r α-Flag, lanes 2 and 5), or rabbit anti-cyclin D1 antibody (r α-D1, lanes 3 and 6). Immunoprecipitates were then immunoblotted with anti-Flag (mα-Flag) and cyclin D1 (m α-D1) mouse antibodies, as indicated. The last lane contains 25 μg of a representative extract from infected insect cells immunoblotted with the same antibodies. **C.** 293 cells were transfected with mammalian expression plasmids encoding HA-tagged β-catenin alone or in combination with either Flag-tagged FBP1 or Flag-tagged (ΔF)FBP1. Cells were lysed and extracts were subjected to immunoprecipitation with a rabbit anti-Flag antibody (r α-Flag, lanes 4-6) and immunoblotted with rat anti-HA (α-HA) and mouse anti-Flag (m α-

Flag) antibodies, as indicated. The first three lanes contain 25 μg of extracts from transfected 293 cells immunoblotted with the same antibodies. Transfecting high levels of β-catenin expression vector, the associations of β-catenin with FBP1 and (ΔF)FBP1 could be determined independently of β-catenin levels.

FIG. 36A-B. Stabilization of β-catenin by a dominant negative (ΔF)FBP1 mutant. **A.** Human 293 cells were transfected with mammalian expression plasmids encoding HA-tagged β-catenin alone or in combination with either Flag-tagged (ΔF)FBP1 or Flag-tagged (ΔF)FBP2. Cells were lysed and extracts were subjected to immunoblotting with rat anti-HA and rabbit anti-Flag (r α-Flag) antibody, as indicated. **B.** Pulse chase analysis of β-catenin turnover rate. HA-tagged β-catenin in combination with either an empty vector, FBP1, or (ΔF)FBP1 was co-transfected in 293 cells. 24 hours later cells were labeled with 35S-methionine for 30 minutes and chased with medium for the indicated times. Extracts were then subjected to immunoprecipitation with a rat anti-HA antibody.

FIG. 37A-C. Binding of phosphorylated p27 to Skp2. **A.** A panel of in vitro translated [35S]FBPs were used in binding reactions with beads coupled to the phospho-peptide NAGSVEQT*PKKPGLRRRQT, corresponding to the carboxy terminus of the human p27 with a phosphothreonine at position 187 (T*). Beads were washed with RIPA buffer and bound proteins were eluted and subjected to electrophoresis and autoradiography (Upper Panel). Bottom Panel: 10% of the in vitro translated [35S]FBP inputs. **B.** HeLa cell extracts were incubated with beads coupled to the phospho-p27 peptide (lane 2), an identical except unphosphorylated p27 peptide (lane 1) or the control phospho-peptide AEIGVGAY*GTVYKARDPHS, corresponding to an amino terminal peptide of human Cdk4 with a phosphotyrosine at position 17 (Y*) (lane 3). Beads were washed with RIPA buffer and bound proteins were immunoblotted with antibodies to the proteins indicated on the left of each panel. A portion of the HeLa extract (25 μg) was used as a control (lane 4). The slower migrating band in Cul1 is likely generated by the covalent attachment of a ubiquitin-like molecule, as already described for other cullins 48. **C.** One μl of in vitro translated [35S] wild type p27 (WT, lanes 1-4) or p27(T187A) mutant (T187A, lanes 5-6) were incubated for 30 minutes at 30½C in 10 μl of kinase buffer. Where indicated, ~2.5 pmole of recombinant purified cyclin E/Cdk2 or ~1 pmole Skp2 (in Skp1/Skp2 complex) were added. Samples were then incubated with 6 μl of Protein-A beads to which antibodies to Skp2 had been covalently linked. Beads were washed with RIPA buffer and bound proteins subjected to electrophoresis and autoradiography. Lanes 1-

6: Skp2-bound proteins; Lanes 7 and 8: 7.5% of the in vitro translated [35S] protein inputs.

FIG. 38. In vivo binding of Skp2 to p27. Extracts from HeLa cells (lanes 1-2 and 5-6) or IMR90 fibroblasts (lanes 9-10) were immunoprecipitated with different affinity purified (AP) antibodies to Skp2 or with purified control IgG fractions. Lane 1: extract immunoprecipitated with a goat IgG (G-IgG); lane 2: with an AP goat antibody to an N-terminal Skp2 peptide (G-α-Skp2,); lanes 5 and 9: with a rabbit IgG (R-IgG); lancs 6 and 10: with an AP rabbit antibody to Skp2 (R-α-Skp2). Immunoprecipitates were immunoblotted with antibodies to the proteins indicated on the left of each panel. Lanes 1-4 in the bottom panel were immunoblotted with a phospho-site p27 specific antibody. Lanes 3, 7, and 11 contain 25 μg of cell extracts; Lanes 4, 8, and 12 contain the relevant recombinant proteins used as markers. The altered migration of some markers is due to the presence of tags on the recombinant proteins.

FIG. 39. Skp2 and cyclin E/Cdk2 complex are rate-limiting for p27 ubiquitination in G1 extracts. a, In vitro ubiquitin ligation (lanes 1-12 and 17-20) and degradation (lanes 13-16) of p27 were carried out with extracts from asynchronously growing (Asyn. ext., lanes 2-3) or G1-arrested (G1 ext., lanes 4-20) HeLa cells. Lane 1 contains no extract. Recombinant purified proteins were supplemented as indicated. Reactions were performed using wild-type p27 (lanes 1-18) or p27(T187A) mutant (T187A, lanes 19-20). Lanes 1-8, 9-12, and 17-20 are from three separate experiments. The bracket on the left side of the panels marks a ladder of bands >27,000 corresponding to polyubiquitinated p27. The asterisk indicates a non-specific band present in most samples. b, Immunoblot analysis of levels of Skp2 and p27 in extracts from asynchronous (lane 1) or G1-arrested (lane 2) HeLa cells.

FIG. 40A-C. Skp2 is required for p27-ubiquitin ligation activity. **A.** Immunodepletion. Extracts from asynchronous HeLa cells were untreated (lane 2) or immunodepleted with pre-immune serum (lane 3), anti-Skp2 antibody pre-incubated with 2 μg of purified GST (lane 4), or anti-Skp2 antibody pre-incubated with 2 μg of purified GST-Skp2 (lane 5). Lane 1 contains no extract. Samples (30 μg of protein) were assayed for p27 ubiquitination in the presence of cyclin E/Cdk2. The bracket on the left side of the panels marks a ladder of bands >27,000 corresponding to polyubiquitinated p27. The asterisk indicates a non-specific band present in all samples. **B.** Reconstitution. The restoration of p27 ubiquitination activity in Skp2-immunodepleted extracts was tested by

the addition of the indicated purified proteins. All samples contained 30 μg of Skp2-depleted extract (Skp2-depl. ext.) and cyclin E/Cdk2. **C.** Immunopurification. Extracts from asynchronous HeLa cells were immunoprecipitated with a rabbit anti-Skp2 antibody (lanes 3 and 5) or pre-immune serum (PI, lanes 2 and 4). Total extract (lane 1) and immuno-beads (lanes 2-5) were added with p27, recombinant purified cyclin E/Cdk2 and ubiquitination reaction mix. Samples in lanes 4 and 5 were supplemented with recombinant purified E1 and Ubc3. All samples were then assayed for p27 ubiquitination.

FIG. 41A-B. In vivo role of Skp2 in p27 degradation. **A.** Stabilization of p27 by a dominant negative (ΔF)Skp2 mutant in vivo. NIH-3T3 cells were transfected with mammalian expression vectors encoding human p27 alone (lane 2), p27 in combination with either (ΔF)Skp2 (lane 3), or (ΔF)FBP1 (lane 4). Lane 1: untransfected cells. Cells were lysed and extracts were subjected to immunoblotting with antibodies to p27, Skp2 or Flag [to detect Flag-tagged (ΔF)FBP1]. Exogenous human p27 protein migrates more slowly than the endogenous murine p27. **B.** Pulse chase analysis of p27 turnover rate. Human p27 in combination with either an empty vector, or (ΔF)Skp2 was transfected in NIH-3T3 cells. Twenty-four hours later, cells were labeled with [35S]-methionine for 20 minutes and chased with medium for the indicated times. Extracts were then subjected to immunoprecipitation with a mouse anti-p27 antibody.

FIG. 42. Stabilization of cellular p27 by antisense oligonucleotides targeting SKP2 mRNA. HeLa cells were treated for 16 -18 hours with two different anti-sense oligodeoxynucleotides (AS) targeting two different regions of SKP2 mRNA. Lanes 2, 6, 12 and 16: AS targeting the N-terminal SKP2 region (NT); Lanes 4 and 8: AS targeting the C-terminal SKP2 region (CT); Lanes 1, 3, 5, 7 11 and 15: control oligodeoxynucleotides pairs (Ctrl). Lanes 1-4, and 5-8 are from two separate experiments. Lanes 11-12 and 15-16: HeLa cells were blocked in G1/S with either Hydroxyurea or Aphidicolin treatment respectively, for 24 hours. Cells were then transfected with oligodeoxynucleotides, lysed after 12 hours (before cells had re-entered G1) and immunoblotted with antibodies to Skp2 (top panels) and p27 (bottom panels). Lanes 9 and 13: Untransfected HeLa cells; Lanes 10 and 14: Untransfected HeLa cells treated with drugs as transfected cells.

FIG. 43A-C. Timing of Skp2 action in the process of p27 degradation. **A.** IMR90 fibroblasts were synchronized in G0/G1 by serum deprivation, reactivated with serum, and sampled at the indicated intervals. Protein extracts were analyzed by immunoblot with the antibodies to the indicated proteins. The Skp2 doublet was likely

generated by phosphorylation since was consistently observed using a 12.5% gel only when cell lysis was performed in the presence of okadaic acid. **B.** HeLa cells blocked in mitosis with nocodazole were shaken off, released in fresh medium and sampled at the indicated intervals. Protein extracts were analyzed by immunoblotting with the antibodies to the indicated proteins. **C.** Extracts from G1 (3 hours after release from nocodazole block) (lane 1) and S-phase (12 hours after release from the nocodazole block) (lane 2) HeLa cells were either immunoprecipitated with an anti-p27 antibody (top two panels) or with an anti-Skp2 antibody (bottom three panels) and then immunoblotted with the antibodies to the indicated proteins.

FIG. 44A-C. Western blot analysis of Skp2/E2F interaction assay. These experiments are described in detail in the Example in Section 8.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel F-box proteins and to novel substrates of F-box proteins. The present invention relates to screening assays designed to identify substrates of the novel F-box proteins and to identify small molecules and compounds which modulate the interaction and/or activity of the F-box proteins and their substrates.

The present invention relates to screening assays to identify substrates of the novel F-box proteins and to identify potential therapeutic agents. The present invention further relates to screening assays based on the identification of novel substrates of both novel and known F-box proteins. The screening assays of the present invention may be used to identify potential therapeutic agents which may be used in protocols and as pharmaceutical compositions designed to target the novel ubiquitin ligases and interactions with their substrates for the treatment of proliferative disorders. In one particular embodiment the present invention relates to screening assays and potential therapeutic agents which target the interaction of FBP with novel substrates β-catenin, p27 and E2F as identified by Applicants.

The invention further encompasses the use of nucleotides encoding the novel F-box proteins, proteins and peptides, as well as antibodies to the novel ubiquitin ligases (which can, for example, act as agonists or antagonists), antagonists that inhibit ubiquitin ligase activity or expression, or agonists that activate ubiquitin ligase activity or increase its expression. In addition, nucleotides encoding the novel ubiquitin ligases and proteins are

useful for the identification of compounds which regulate or mimic their activity and therefore are potentially effective in the treatment of cancer and tumorigenesis.

In particular, the invention described in the subsections below encompasses FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 polypeptides or peptides corresponding to functional domains of the novel ubiquitin ligases (*e.g.*, the F-box motif, the substrate binding domain, and leucine-rich repeats), mutated, truncated or deleted (*e.g.* with one or more functional domains or portions thereof deleted), ubiquitin ligase fusion proteins, nucleotide sequences encoding such products, and host cell expression systems that can produce such ubiquitin ligase products.

The present invention provides methods of screening for peptides and proteins that interact with novel components of the ubiquitin ligase complex, including FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 or derivatives, fragments or analogs thereof. Preferably, the method of screening is a yeast two-hybrid assay system or a variation thereof, as further described below. Derivatives (*e.g.*, fragments) and analogs of a protein can be assayed for binding to a binding partner by any method known in the art, for example, the modified yeast two-hybrid assay system described below, immunoprecipitation with an antibody that binds to the protein in a complex followed by analysis by size fractionation of the immunoprecipitated proteins (*e.g.*, by denaturing or nondenaturing polyacrylamide gel electrophoresis), Western analysis, non-denaturing gel electrophoresis, *etc.*

The present invention relates to screening assays to identify agents which modulate the activity of the novel ubiquitin ligases. The invention encompasses both in vivo and in vitro assays to screen small molecules, compounds, recombinant proteins, peptides, nucleic acids, antibodies *etc.* which modulate the activity of the novel ubiquitin ligases and thus, identify potential therapeutic agents for the treatment of proliferative or differentiative disorders. In one embodiment, the present invention provides methods of screening for proteins that interact with the novel ubiquitin ligases.

The invention also encompasses antibodies and anti-idiotypic antibodies, antagonists and agonists, as well as compounds or nucleotide constructs that inhibit expression of the ubiquitin ligase gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of the ubiquitin ligase (*e.g.*, expression constructs in which ubiquitin ligase coding sequences are operatively associated with expression control elements such as

promoters, promoter/enhancers, *etc.*). The invention also relates to host cells and animals genetically engineered to express the human (or mutants thereof) or to inhibit or "knock-out" expression of the animal's endogenous ubiquitin ligase.

Finally, the ubiquitin ligase protein products and fusion protein products, (*i.e.*, fusions of the proteins or a domain of the protein, *e.g.*, F-box motif), antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including compounds that modulate the ubiquitization pathway can be used for therapy of proliferative or differentiative diseases. Thus, the invention also encompasses pharmaceutical formulations and methods for treating cancer and tumorigenesis.

Various aspects of the invention are described in greater detail in the subsections below.

5.1 FBP GENES

The invention provides nucleic acid molecules comprising seven novel nucleotide sequences, and fragments thereof, FBP1, FBP2, FBP3a, FBP4, FBP5, FBP6, and FBP7, nucleic acids which are novel genes identified by the interaction of their gene products with Skp1, a component of the ubiquitin ligase complex. The invention further provides fourteen novel nucleic acid molecules comprising the nucleotide sequences of FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP11, FBP12, FBP13, FBP14, FBP15, FBP17, FBP18, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, which Nucleic acid sequences of the identified FBP genes are described herein.

As used herein, "an FBP gene" refers to:

(a) a nucleic acid molecule containing the DNA sequences of FBP1, shown in Figure 3 (SEQ ID NO:1), the DNA sequences of FBP2, shown in Figure 4 (SEQ ID NO:3), the DNA sequences of FBP3a, shown in Figure 5 (SEQ ID NO:5), the DNA sequences of FBP3b, shown in Figure 6 (SEQ ID NO:23), the DNA sequences of FBP4, shown in Figure 7 (SEQ ID NO:7), the DNA sequences of FBP5, shown in Figure 8 (SEQ ID NO:9), the DNA sequences of FBP6, shown in Figure 9 (SEQ ID NO:11), the DNA sequences of FBP7, shown in Figure 10 (SEQ ID NO:13), the DNA sequences of FBP8, shown in Figure 11 (SEQ ID NO:25), the DNA sequences of FBP9, shown in Figure 12 (SEQ ID NO:27), the DNA sequences of FBP10, shown in Figure 13 (SEQ ID NO:29), the DNA sequences of FBP11, shown in Figure 14 (SEQ ID NO:31), the DNA sequences of FBP12, shown in Figure 15 (SEQ ID NO:33), the DNA sequences of FBP13, shown in Figure 16 (SEQ ID NO:35), the DNA sequences of FBP14, shown in Figure 17 (SEQ ID NO:37), the DNA sequences of FBP15, shown in Figure 18 (SEQ ID NO:39), the DNA sequences of FBP16, shown in Figure 19 (SEQ ID NO:41), the DNA sequences of FBP17,

shown in Figure 20 (SEQ ID NO:43), the DNA sequences of FBP18, shown in Figure 21 (SEQ ID NO:45), the DNA sequences of FBP19, shown in Figure 22 (SEQ ID NO:47), the DNA sequences of FBP20, shown in Figure 23 (SEQ ID NO:49), the DNA sequences of FBP21, shown in Figure 24 (SEQ ID NO:51), the DNA sequences of FBP22, shown in Figure 25 (SEQ ID NO:53), the DNA sequences of FBP23, shown in Figure 26 (SEQ ID NO:55), the DNA sequences of FBP24, shown in Figure 27 (SEQ ID NO:57), the DNA sequences of FBP25, shown in Figure 28 (SEQ ID NO:59).

(b) any DNA sequence that encodes a polypeptide containing: the amino acid sequence of FBP1 shown in Figure 3A (SEQ ID NO:2), the amino acid sequence of FBP2, shown in Figure 4A (SEQ ID NO:4), the amino acid sequence of FBP3a shown in Figure 5A (SEQ ID NO:6), the amino acid sequence of FBP3b shown in Figure 6A (SEQ ID NO:24), the amino acid sequence of FBP4 shown in Figure 7A (SEQ ID NO:8), the amino acid sequence of FBP5 shown in Figure 8A (SEQ ID NO:10), or the amino acid sequence of FBP6 shown in Figure 9A (SEQ ID NO:12), the amino acid sequences of FBP7, shown in Figure 10 (SEQ ID NO:14), the amino acid sequences of FBP8, shown in Figure 11 (SEQ ID NO:26), the amino acid sequences of FBP9, shown in Figure 12 (SEQ ID NO:28), the amino acid sequences of FBP10, shown in Figure 13 (SEQ ID NO:30), the amino acid sequences of FBP11, shown in Figure 14 (SEQ ID NO:32), the amino acid sequences of FBP12, shown in Figure 15 (SEQ ID NO:34), the amino acid sequences of FBP13, shown in Figure 16 (SEQ ID NO:36), the amino acid sequences of FBP14, shown in Figure 17 (SEQ ID NO:38), the amino acid sequences of FBP15, shown in Figure 18 (SEQ ID NO:40), the amino acid sequences of FBP16, shown in Figure 19 (SEQ ID NO:42), the amino acid sequences of FBP17, shown in Figure 20 (SEQ ID NO:44), the amino acid sequences of FBP18, shown in Figure 21 (SEQ ID NO:46), the amino acid sequences of FBP19, shown in Figure 22 (SEQ ID NO:48), the amino acid sequences of FBP20, shown in Figure 23 (SEQ ID NO:50), the amino acid sequences of FBP21, shown in Figure 24 (SEQ ID NO:52), the amino acid sequences of FBP22, shown in Figure 25 (SEQ ID NO:54), the amino acid sequences of FBP23, shown in Figure 26 (SEQ ID NO:56), the amino acid sequences of FBP24, shown in Figure 27 (SEQ ID NO:58), the amino acid sequences of FBP25, shown in Figure 28 (SEQ ID NO:60).

(c) any DNA sequence that hybridizes to the complement of the DNA sequences that encode any of the amino acid sequences of (SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14) or Figure 15 under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65 C, and washing in 0.1xSSC/0.1% SDS at 68 C (Ausubel F.M. et al., eds., 1989, Current Protocols in

Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3); and/or

(d) any DNA sequence that hybridizes to the complement of the DNA sequences that encode any of the amino acid sequences in (SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14) or Figure 15, under less stringent conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2xSSC/0.1% SDS at 42 C (Ausubel et al., 1989, *supra*), and encodes a gene product functionally equivalent to an FBP gene product.

It is understood that the FBP gene sequences of the present invention do not encompass the previously described genes encoding other mammalian F-box proteins, Skp2, Elongin A, Cyclin F, mouse Md6, (see Pagano, 1997, *supra*; Zhang et al., 1995, *supra*; Bai et al., 1996, *supra*; Skowyra et al., 1997, *supra*). It is further understood that the nucleic acid molecules of the invention do not include nucleic acid molecules that consist solely of the nucleotide sequence in GenBank Accession Nos. AC002428, AI457595, AI105408, H66467, T47217, H38755, THC274684, AI750732, AA976979, AI571815, T57296, Z44228, Z45230, N42405, AA018063, AI751015, AI400663, T74432, AA402415, AI826000, AI590138, AF174602, Z45775, AF174599, THC288870, AI017603, AF174598, THC260994, AI475671, AA768343, AF174595, THC240016, N70417, T10511, AF174603, EST04915, AA147429, AI192344, AF174594, AI147207, AI279712, AA593015, AA644633, AA335703, N26196, AF174604, AF053356, AF174606, AA836036, AA853045, AI479142, AA772788, AA039454, AA397652, AA463756, AA007384, AA749085, AI640599, THC253263, AB020647, THC295423, AA434109, AA370939, AA215393, THC271423, AF052097, THC288182, AL049953, CAB37981, AL022395, AL031178, THC197682, and THC205131.

FBP sequences of the present invention are derived from a eukaryotic genome, preferably a mammalian genome, and more preferably a human or murine genome. Thus, the nucleotide sequences of the present invention do not encompass those derived from yeast genomes. In a specific embodiment, the nucleotides of the present invention encompass any DNA sequence derived from a mammalian genome which hybridizes under highly stringent conditions to SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13, or to DNA sequence shown in Figure 14, encodes a gene product which contains an F-box motif and binds to Skp1. In a specific embodiment, the nucleotides of the present invention encompass any DNA sequence derived from a mammalian genome which hybridize under highly stringent conditions to SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13 encodes a gene product which contains an F-box motif and another domain selected from the group comprising WD-40, leucine rich region, leucine zipper motif, or other protein-protein interaction domain, and binds to Skp-1 and is at least 300 or 400 nucleotides in length.

FBP sequences can include, for example, either eukaryotic genomic DNA (cDNA) or cDNA sequences. When referring to a nucleic acid which encodes a given amino acid sequence, therefore, it is to be understood that the nucleic acid need not only be a cDNA molecule, but can also, for example, refer to a cDNA sequence from which an mRNA species is transcribed that is processed to encode the given amino acid sequence.

As used herein, an FBP gene may also refer to degenerate variants of DNA sequences (a) through (d).

The invention also includes nucleic acid molecules derived from mammalian nucleic acids, preferably DNA molecules, that hybridize to, and are therefore the complements of, the DNA sequences (a) through (d), in the preceding paragraph. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, *e.g.*, to washing in 6xSSC/0.05% sodium pyrophosphate at 37 C (for 14-base oligos), 48 C (for 17-base oligos), 55 C (for 20-base oligos), and 60 C (for 23-base oligos). These nucleic acid molecules may encode or act as FBP gene antisense molecules, useful, for example, in FBP gene regulation (for and/or as antisense primers in amplification reactions of FBP gene nucleic acid sequences). With respect to FBP gene regulation, such techniques can be used to regulate, for example, an FBP-regulated pathway, in order to block cell proliferation associated with cancer. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for FBP gene regulation. Still further, such molecules may be used as components of diagnostic methods whereby, for example, the presence of a particular FBP allele responsible for causing an FBP-related disorder, *e.g.*, proliferative or differentiative disorders such as tumorigenesis or cancer, may be detected.

The invention also encompasses:

(a) DNA vectors that contain any of the foregoing FBP coding sequences and/or their complements (*i.e.*, antisense);

(b) DNA expression vectors that contain any of the foregoing FBP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and

(c) genetically engineered host cells that contain any of the foregoing FBP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell.

As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but

are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast-mating factors.

The invention further includes fragments of any of the DNA sequences disclosed herein.

In one embodiment, the FBP gene sequences of the invention are mammalian gene sequences, with human sequences being preferred.

In yet another embodiment, the FBP gene sequences of the invention are gene sequences encoding FBP gene products containing polypeptide portions corresponding to (that is, polypeptide portions exhibiting amino acid sequence similarity to) the amino acid sequence depicted in Figures 2, 4-9 or 15, wherein the corresponding portion exhibits greater than about 50% amino acid identity with the depicted sequence, averaged across the FBP gene product's entire length.

In specific embodiments, F-box encoding nucleic acids comprise the cDNA sequences of SEQ ID NOs: 1, 3, 5, 23, 7, 9, 11, 13, 15, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, or 59, nucleotide sequence of Figures 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, 11B, 12B, 13B, 14B, 15B, 16B, 17B, 18B, 19B, 20B, 21B, 22B, 23B, 24B, 25B, 26B, 27B, or 28B, respectively, or the coding regions thereof, or nucleic acids encoding an F-box protein (*e.g.*, a protein having the sequence of SEQ ID NOs: 2, 4, 6, 24, 8, 10, 12, 14, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 68, or 60, or as shown in Figures 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 11A, 12A, 13A, 14A, 15A, 16A, 17A, 18A, 19A, 20A, 21A, 22A, 23A, 24A, 25A, 26A, 27A, or 28A, respectively).

The invention further provides nucleotide fragments of nucleotide sequences encoding FBP1, FBP2, FBP3a, FBP4, FBP5, FBP6, or FBP7 (SEQ ID NOs: 1, 3, 5, 7, 9, 11 and 13, respectively) of the invention. Such fragments consist of at least 8 nucleotides (*i.e.*, a hybridizable portion) of an FBP gene sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of an F-box sequence, or a full-length F-box coding sequence. In another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. Nucleic acids can be single or double stranded. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of an F-box gene.

The invention further relates to the human genomic nucleotide sequences of nucleic acids. In specific embodiments, F-box encoding nucleic acids comprise the genomic sequences of SEQ ID NOs:1, 3, 5, 7, 9, 11 or 13 or the coding regions thereof, or nucleic acids encoding an FBP protein (*e.g.*, a protein having the sequence of SEQ ID Nos: 2, 4, 6, 8, 10, 12 or 14). The invention provides purified nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of an FBP gene sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of an FBP gene sequence or a full-length FBP gene coding sequence. In another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. Nucleic acids can be single or double stranded. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of an FBP gene sequence.

In addition to the human FBP nucleotide sequences disclosed herein, other FBP gene sequences can be identified and readily isolated, without undue experimentation, by molecular biological techniques well known in the art, used in conjunction with the FBP gene sequences disclosed herein. For example, additional human FBP gene sequences at the same or at different genetic loci as those disclosed in SEQ ID NOs:1, 3, 5, 7, 9, 11 or 13 can be isolated readily. There can exist, for example, genes at other genetic or physical loci within the human genome that encode proteins that have extensive homology to one or more domains of the FBP gene products and that encode gene products functionally equivalent to an FBP gene product. Further, homologous FBP gene sequences present in other species can be identified and isolated readily.

The FBP nucleotide sequences of the invention further include nucleotide sequences that encode polypeptides having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or higher amino acid sequence identity to the polypeptides encoded by the FBP nucleotide sequences of SEQ ID No.1, 3, 5, 7, 9, 11 or 13.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the

molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical overlapping positions/total # of overlapping positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al., 1990, J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.25*:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Altschul et al., 1997, *supra*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al., 1990, J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.25*:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Altschul et al., 1997, *supra*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical

algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS 4*:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

With respect to identification and isolation of FBP gene sequences present at the same genetic or physical locus as those sequences disclosed herein, such sequences can, for example, be obtained readily by utilizing standard sequencing and bacterial artificial chromosome (BAC) technologies.

With respect to the cloning of an FBP gene homologue in human or other species (*e.g.*, mouse), the isolated FBP gene sequences disclosed herein may be labeled and used to screen a cDNA library constructed from mRNA obtained from appropriate cells or tissues (*e.g.*, brain tissues) derived from the organism (*e.g.*, mouse) of interest. The hybridization conditions used should be of a lower stringency when the cDNA library is derived from an organism different from the type of organism from which the labeled sequence was derived.

Alternatively, the labeled fragment may be used to screen a genomic library derived from the organism of interest, again, using appropriately stringent conditions. Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y.; and Ausubel, et al., supra. Further, an FBP gene homologue may be isolated from, for example, human nucleic acid, by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of amino acid sequences within any FBP gene product disclosed herein.

The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences of an FBP gene nucleic acid sequence. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to isolate genomic clones via the screening of a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source (*i.e.*, one known, or suspected, to express the FBP gene, such as, for example, blood samples or brain tissue samples obtained through biopsy or post-mortem). A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies that may be used, see *e.g.*, Sambrook et al., supra.

FBP gene sequences may additionally be used to identify mutant FBP gene alleles. Such mutant alleles may be isolated from individuals either known or proposed to have a genotype that contributes to the symptoms of an FBP gene disorder, such as proliferative or differentiative disorders involved in tumorigenesis or causing cancer, for example. Mutant alleles and mutant allele products may then be utilized in the therapeutic, diagnostic and prognostic systems described below. Additionally, such FBP gene sequences can be used to detect FBP gene regulatory (*e.g.*, promoter) defects which can be associated with an FBP disorder, such as proliferative or differentiative disorders involved in tumorigenesis or causing cancer, for example.

FBP alleles may be identified by single strand conformational polymorphism (SSCP) mutation detection techniques, Southern blot, and/or PCR amplification techniques. Primers can routinely be designed to amplify overlapping regions of the whole FBP sequence including the promoter region. In one embodiment, primers are designed to cover the exon-intron boundaries such that, first, coding regions can be scanned for mutations. Genomic DNA isolated from lymphocytes of normal and affected individuals is used as PCR template. PCR products from normal and affected individuals are compared, either by single strand conformational polymorphism (SSCP) mutation detection techniques and/or by sequencing. SSCP analysis can be performed as follows: 100 ng of genomic DNA is amplified in a 10 1 reaction, adding 10 pmols of each primer, 0.5 U of Taq DNA polymerase (Promega), 1 Ci of -[32P]dCTP (NEN; specific activity, 3000 Ci/mmol), in 2.5 M dNTPs (Pharmacia), 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1 mM MgCl2, 0.01% gelatin, final concentration. Thirty cycles of denaturation (94°C), annealing (56°C to 64°C, depending on primer melting temperature), and extension (72°C) is carried out in a thermal-cycler (MJ Research, Boston, MA, USA), followed by a 7 min final extension at 72°C. Two microliters of the reaction mixture is diluted in 0.1% SDS, 10 mM EDTA and

then mixed 1: 1 with a sequencing stop solution containing 20 mM NaOH. Samples are heated at 95 C for 5 min, chilled on ice for 3 min and then 3 1 will be loaded onto a 6% acrylamide/TBE gel containing 5% (v/v) glycerol. Gels are run at 8 W for 12-15 h at room temperature. Autoradiography is performed by exposure to film at -70 C with intensifying screens for different periods of time. The mutations responsible for the loss or alteration of function of the mutant FBP gene product can then be ascertained.

Alternatively, a cDNA of a mutant FBP gene may be isolated, for example, using PCR. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying the mutant FBP allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant FBP allele to that of the normal FBP allele, the mutation(s) responsible for the loss or alteration of function of the mutant FBP gene product can be ascertained.

Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry a mutant FBP allele, or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant FBP allele. An unimpaired FBP gene or any suitable fragment thereof may then be labeled and used as a probe to identify the corresponding mutant FBP allele in such libraries. Clones containing the mutant FBP gene sequences may then be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant FBP allele in an individual suspected of or known to carry such a mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal FBP gene product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

Nucleic acids encoding derivatives and analogs of FBP proteins, and FBP antisense nucleic acids can be isolated by the methods recited above. As used herein, a "nucleic acid encoding a fragment or portion of an F-box protein" shall be construed as

referring to a nucleic acid encoding only the recited fragment or portion of the FBP and not the other contiguous portions of the FBP protein as a continuous sequence.

Fragments of FBP gene nucleic acids comprising regions conserved between (*i.e.*, with homology to) other FBP gene nucleic acids, of the same or different species, are also provided. Nucleic acids encoding one or more FBP domains can be isolated by the methods recited above.

In cases where an FBP mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), a polyclonal set of anti-FBP gene product antibodies are likely to cross-react with the mutant FBP gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

5.2 PROTEINS AND POLYPEPTIDES OF FBP GENES

The amino acid sequences depicted in Figures 1, 2, and parts B of Figures 3 to 28 represent FBP gene products. The FBP1 gene product, sometimes referred to herein as a "FBP1 protein", includes those gene products encoded by the FBP1 gene sequences described in Section 5.1, above. Likewise, the FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 gene products, referred to herein as an FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 proteins, include those gene products encoded by the FBP2, FBP3, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25 genes. In accordance with the present invention, the nucleic acid sequences encoding the FBP gene products are derived from eukaryotic genomes, including mammalian genomes. In a preferred embodiment the nucleic acid sequences encoding the FBP gene products are derived from human or murine genomes.

FBP gene products, or peptide fragments thereof, can be prepared for a variety of uses. For example, such gene products, or peptide fragments thereof, can be used for the generation of antibodies, in diagnostic and prognostic assays, or for the identification of other cellular or extracellular gene products involved in the ubiquitination pathway and thereby implicated in the regulation of cell cycle and proliferative disorders.

In addition, FBP gene products of the present invention may include proteins that represent functionally equivalent (see Section 5.1 for a definition) gene products. FBP

gene products of the invention do not encompass the previously identified mammalian F-box proteins Skp2, Cyclin F, Elongin A, or mouse Md6 (see Pagano, 1997, supra; Zhang et al., 1995 supra; Bai et al., 1996 supra; Skowyra et al., 1997, supra).

Functionally equivalent FBP gene products may contain deletions, including internal deletions, additions, including additions yielding fusion proteins, or substitutions of amino acid residues within and/or adjacent to the amino acid sequence encoded by the FBP gene sequences described, above, in Section 5.1, but that result in a "silent" change, in that the change produces a functionally equivalent FBP gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Alternatively, where alteration of function is desired, deletion or non-conservative alterations can be engineered to produce altered FBP gene products. Such alterations can, for example, alter one or more of the biological functions of the FBP gene product. Further, such alterations can be selected so as to generate FBP gene products that are better suited for expression, scale up, *etc.* in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The FBP gene products, peptide fragments thereof and fusion proteins thereof, may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the FBP gene polypeptides, peptides, fusion peptide and fusion polypeptides of the invention by expressing nucleic acid containing FBP gene sequences are described herein. Methods that are well known to those skilled in the art can be used to construct expression vectors containing FBP gene product coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook, et al., supra, and Ausubel, et al., supra. Alternatively, RNA capable of encoding FBP gene product sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, ed., IRL Press, Oxford.

A variety of host-expression vector systems may be utilized to express the FBP gene coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells that may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the FBP gene product of the invention in situ. These include but are not limited to microorganisms such as bacteria (*e.g.*, E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing FBP gene product coding sequences; yeast (*e.g.*, Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the FBP gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the FBP gene product coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing FBP gene product coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the FBP gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of FBP protein or for raising antibodies to FBP protein, for example, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2, 1791), in which the FBP gene product coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, 1985, Nucleic Acids Res. 13, 3101-3109; Van Heeke and Schuster, 1989, J. Biol. Chem. 264, 5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica, nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera

frugiperda cells. The FBP gene coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of FBP gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed (*e.g.*, see Smith, et al., 1983, J. Virol. 46, 584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells. a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the FBP gene coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing *FBP* gene product in infected hosts. (*e.g.*, See Logan and Shenk, 1984, Proc. Natl. Acad. Sci. USA 81, 3655-3659). Specific initiation signals may also be required for efficient translation of inserted FBP gene product coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire FBP gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the FBP gene coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (see Bittner, et al., 1987, Methods in Enzymol. 153, 516-544).

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing

of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the FBP gene product may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the FBP gene product. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the *FBP* gene product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48, 2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22, 817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77, 3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78, 1527); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, Proc. Natl. Acad. Sci. USA 78, 2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150, 1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30, 147).

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht, et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88, 8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with

recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The FBP gene products can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, sheep, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate FBP transgenic animals. The term "transgenic," as used herein, refers to animals expressing FBP gene sequences from a different species (*e.g.*, mice expressing human FBP sequences), as well as animals that have been genetically engineered to overexpress endogenous (*i.e.*, same species) FBP sequences or animals that have been genetically engineered to no longer express endogenous FBP gene sequences (*i.e.*, "knock-out" animals), and their progeny.

In particular, the present invention relates to FBP1 knockout mice. The present invention also relates to transgenic mice which express human wild-type FBP1 and Skp2 gene sequences in addition to mice engineered to express human mutant FBP1 and Skp2 gene sequences deleted of their F-box domains. Any technique known in the art may be used to introduce an FBP gene transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe and Wagner, 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten, et al., 1985, Proc. Natl. Acad. Sci., USA 82, 6148-6152); gene targeting in embryonic stem cells (Thompson, et al., 1989, Cell 56, 313-321); electroporation of embryos (Lo, 1983, Mol. Cell. Biol. 3, 1803-1814); and sperm-mediated gene transfer (Lavitrano et al., 1989, Cell 57, 717-723) (For a review of such techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115, 171-229)

Any technique known in the art may be used to produce transgenic animal clones containing an FBP transgene, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal or adult cells induced to quiescence (Campbell, et al., 1996, Nature 380, 64-66; Wilmut, et al., Nature 385, 810-813).

The present invention provides for transgenic animals that carry an FBP transgene in all their cells, as well as animals that carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, et al., 1992, Proc. Natl. Acad. Sci. USA 89, 6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. Examples of regulatory sequences that can be used to direct tissue-specific expression

of an FBP transgene include, but are not limited to, the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:42S-51S); the insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122); immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adams *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444): albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276) alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58); alpha-1-antitrypsin gene control region which is active in liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171); beta-globin gene control region which is active in myeloid cells (Magram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286); and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378). Promoters isolated from the genome of viruses that grow in mammalian cells, (*e.g.*, vaccinia virus 7.5K, SV40, HSV, adenoviruses MLP, MMTV, LTR and CMV promoters) may be used, as well as promoters produced by recombinant DNA or synthetic techniques.

When it is desired that the FBP gene transgene be integrated into the chromosomal site of the endogenous FBP gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous FBP gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous FBP gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous FBP gene in only that cell type, by following, for example, the teaching of Gu, et al. (Gu, et al., 1994, Science 265, 103-106). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant FBP gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using

techniques that include but are not limited to Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR (reverse transcriptase PCR). Samples of FBP gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the FBP transgene product.

Transgenic mice harboring tissue-directed transgenes can be used to test the effects of FBP gene expression the intact animal. In one embodiment, transgenic mice harboring a human FBP1 transgene in the mammary gland can be used to assess the role of FBPs in mouse mammary development and tumorigenesis. In another embodiment, transgenic mice can be generated that overexpress the human FBP1 dominant negative mutant form (F-box deleted) in the mammary gland. In a specific embodiment, for example, the MMTV LTR promoter (mouse mammary tumor virus long terminal repeat) can be used to direct integration of the transgene in the mammary gland. An MMTV/FBP1 fusion gene can be constructed by fusing sequences of the MMTV LTR promoter to nucleotide sequences upstream of the first ATG of FBP1 gene. An SV40 polyadenylation region can also be fused to sequences downstream of the FBP1 coding region. Transgenic mice are generated by methods well known in the art (Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115, 171-229). Briefly, immature B6D2F1 female mice are superovulated and mated to CD-1 males. The following morning the females are examined for the presence of vaginal plugs, and fertilized ova are recovered and microinjected with a plasmid vector. Approximately 2000 copies of the material are microinjected into each pronucleus. Screening of founder animals is performed by extraction of DNA from spleen and Southern hybridization using the MMTV/FBP1 as a probe. Screening of offspring is performed by PCR of tail DNA. Once transgenic pedigrees are established, the expression pattern of the transgene is determined by Northern blot and RT-PCR analysis in different organs in order to correlate it with subsequent pathological changes.

The resulting transgenic animals can then be examined for the role of FBP genes in tumorigenesis. In one embodiment, for example, FBP transgenes can be constructed for use as a breast cancer model. Overexpression of FBP1 genes in such mice is expected to increase β-catenin ubiquitination and degradation, resulting in a tumor suppressor phenotype. Conversely, overexpression of the FBP1 deletion mutant is expected to result in stabilization of β-catenin and induce proliferation of mammary gland epithelium. These phenotypes can be tested in both female and male transgenic mice, by assays such as those described in Sections 5.4, 5.5 and 7.

In another specific embodiment, transgenic mice are generated that express FBP1 transgenes in T-lymphocytes. In this embodiment, a CD2/FBP1 fusion gene is constructed by fusion of the CD2 promoter, which drives expression in both CD4 positive

and negative T-cells, to sequences located upstream of the first ATG of an FBP gene, e.g., the wild-type and mutant FBP1 genes. The construct can also contain an SV40 polyadenylation region downstream of the FBP gene. After generation and testing of transgenic mice, as described above, the expression of the FBP transgene is examined. The transgene is expressed in thymus and spleen. Overexpression of wild-type FBP1 is expected to result in a phenotype. For example, possible expected phenotypes of FBP1 transgenic mice include increased degradation of IKBα, increased activation of NFKB, or increased cell proliferation. Conversely, overexpression of the dominant negative mutant, FBP1, lacking the F-box domain, can be expected to have the opposite effect, for example, increased stability of IKBα, decreased activation of NFKB, or decreased cell proliferation. Such transgenic phenotypes can be tested by assays such as those used in Section 5.4 and 5.5.

In another specific embodiment, the SKP2 gene is expressed in T-lymphocytes of trangenic mice. Conversely, the F-box deletion form acts as dominant negative, stabilizing p27 and inhibiting T-cell activation. Construction of the CD2/SKP2 fusion genes and production of transgenic mice are as described above for CD2/ FBP fusion genes, using wild-type and mutant SKP2 cDNA, instead of FBP1 cDNA, controlled by the CD2 promoter. Founders and their progeny are analyzed for the presence and expression of the SKP2 transgene and the mutant SKP2 transgene. Expression of the transgene in spleen and thymus is analyzed by Northern blot and RT-PCR

In another specific embodiment, transgenic mice are constructed by inactivation of the FBP1 locus in mice. Inactivation of the FBP1 locus in mice by homologous recombination involves four stages: 1) the construction of the targeting vector for FBP1; 2) the generation of ES +/- cells; 3) the production of knock-out mice; and 4) the characterization of the phenotype. A 129 SV mouse genomic phage library is used to identify and isolate the mouse FBP1 gene. Bacteriophages are plated at an appropriate density and an imprint of the pattern of plaques can be obtained by gently layering a nylon membrane onto the surface of agarose dishes. Bacteriophage particles and DNA are transferred to the filter by capillary action in an exact replica of the pattern of plaques. After denaturation, the DNA is bound to the filter by baking and then hybridized with ^{32}P-labeled-FBP1 cDNA. Excess probe is washed away and the filters were then exposed for autoradiography. Hybridizing plaques, identified by aligning the film with the original agar plate, were picked for a secondary and a tertiary screening to obtain a pure plaque preparation. Using this method, positive phage which span the region of interest, for example, the region encoding the F-box, are isolated. Using PCR, Southern hybridization,

restriction mapping, subcloning and DNA sequencing the partial structure of the wild-type FBP1 gene can be determined.

To inactivate the Fbp1 locus by homologous recombination, a gene targeting vector in which exon 3 in the Fbp1 locus is replaced by a selectable marker, for example, the neoR gene, in an antisense orientation can be constructed. Exon 3 encodes the F-box motif which is known to be critical for Fbp1 interaction with Skp1. The targeting construct possesses a short and a long arm of homology flanking a selectable marker gene. One of the vector arms is relatively short (2 kb) to ensure efficient amplification since homologous recombinant ES clones will be screened by PCR. The other arm is >6 kb to maximize the frequency of homologous recombination. A thymidine kinase (tk) gene, included at the end of the long homology arm of the vector provides an additional negative selection marker (using ganeylovir) against ES clones which randomly integrate the targeting vector. Since homologous recombination occurs frequently using linear DNA, the targeting vector is linearized prior to transfection of ES cells. Following electroporation and double drug selection of embryonic stem cell clones, PCR and Southern analysis is used to determine whether homologous recombination has occurred at the FBP1 locus. Screening by PCR is advantageous because a larger number of colonies can be analyzed with this method than with Southern analysis. In addition, PCR screening allows rapid elimination of negative clones thus to avoid feeding and subsequently freezing all the clones while recombinants are identified. This PCR strategy for detection of homologous recombinants is based on the use of a primer pair chosen such that one primer anneals to a sequence specific to the targeting construct, *e.g.*, sequences of the neomycin gene or other selectable marker, and not in the endogenous locus, and the other primer anneals to a region outside the construct, but within the endogenous locus. Southern analysis is used to confirm that a homologous recombination event has occurred (both at the short arm of homology and at the long arm of homology) and that no gene duplication events have occurred during the recombination.

Such FBP1 knockout mice can be used to test the role of Fbp1 in cellular regulation and control of proliferation. In one embodiment, phenotype of such mice lacking Fbp1 is cellular hyperplasia and increased tumor formation. In another embodiment, FBP1 null mice phenotypes include, but are not limited to, increased β-catenin activity, stabilization of β-catenin, increased cellular proliferation, accumulation of IK-Ba, decreased NF-KB activity, deficient immune response, inflammation, or increased cell death or apoptotic activity. Alternatively, a deletion of the of the FBP1 gene can result in an embryonic lethality. In this case, heterozygous mice at the FBP1 allele can be tested using

the above assays, and embryos of null FBP mice can be tested using the assays described above.

Transgenic mice bearing FBP transgenes can also be used to screen for compounds capable of modulating the expression of the FBP gene and/or the synthesis or activity of the FBP1 gene or gene product. Such compounds and methods for screening are described.

5.3 GENERATION OF ANTIBODIES TO F-BOX PROTEINS AND THEIR DERIVATIVES

According to the invention, F-box motif, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to a human FBP protein are produced. In another embodiment, antibodies to a domain (*e.g.*, the F-box domain or the substrate-binding domain) of an FBP are produced.

Various procedures known in the art may be used for the production of polyclonal antibodies to an FBP or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of an FBP encoded by a sequence of FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, or a subsequence thereof, can be obtained (Pagano, M., 1995, "From peptide to purified antibody", in Cell Cycle: Materials and Methods. M. Pagano, ed. Spring-Verlag. 217-281). For the production of antibody, various host animals can be immunized by injection with the native FBP, or a synthetic version, or derivative (*e.g.*, fragment) thereof, including but not limited to rabbits, mice, rats, *etc.* Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward an FBP sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the

trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for FBP together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce FBP-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for FBPs, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent, and Fv fragments.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of an FBP, one may assay generated hybridomas for a product which binds to an FBP fragment containing such domain. For selection of an antibody that specifically binds a first FBP homolog but which does not specifically bind a different FBP homolog, one can select on the basis of positive binding to the first FBP homolog and a lack of binding to the second FBP homolog.

Antibodies specific to a domain of an FBP are also provided, such as an F-box motif.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the FBP sequences of the invention, *e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, *etc.*

In another embodiment of the invention (see *infra*), anti-FBP antibodies and fragments thereof containing the binding domain are used as therapeutics.

5.4 SCREENING ASSAYS FOR THE IDENTIFICATION OF AGENTS THAT INTERACT WITH F-BOX PROTEINS AND/OR INTERFERE WITH THEIR ENZYMATIC ACTIVITIES

Novel components of the ubiquitin ligase complex, including FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25, interact with cellular proteins to regulate cellular proliferation. One aspect of the present invention provides methods for assaying and screening fragments, derivatives and analogs of the novel components to identify polypeptides or peptides or other compounds that interact with the novel ubiquitin ligases such as potential substrates of ubiquitin ligase activity. The present invention also provides screening assays to identify compounds that modulate or inhibit the interaction of the novel FBPs with other subunits or numbers of the ubiquitin ligase complex, such as Skp1, or ubiquitinating enzymes with which the novel FBPs interact.

In yet another embodiment, the assays of the present invention may be used to identify polypeptides or peptides or other compounds which inhibit or modulate the interaction between the novel ubiquitin ligases or known (*e.g.*, Skp1) components of the ubiquitin ligase complex with novel or known substrates. By way of example, but not by limitation, the screening assays described herein may be used to identify peptides or proteins that interfere with the interaction between known ubiquitin ligase component, Skp2, and its novel substrate, p27. In another example, compounds that interfere with the interaction between FBP1 and its novel substrate, β-catenin, are identified using the screening assay. In another example, compounds that interfere with the interaction between Skp2 and another putative substrate, E2F, are identified using the screening assay. In yet another example, compounds that interfere with the interaction between FBP1 and another putative substrate, IKBα, are identified using the screening assay.

In yet another embodiment, the assays of the present invention may be used to identify polypeptides or peptides which inhibit or activate the enzymatic activators of the novel FBPs.

5.4.1 ASSAYS FOR PROTEIN-PROTEIN INTERACTIONS

Derivatives, analogs and fragments of proteins that interact with the novel components of the ubiquitin ligase complex of the present invention can be identified by means of a yeast two hybrid assay system (Fields and Song, 1989, Nature 340:245-246 and U.S. Patent No. 5,283,173). Because the interactions are screened for in yeast, the intermolecular protein interactions detected in this system occur under physiological conditions that mimic the conditions in mammalian cells (Chien et al., 1991, Proc. Natl. Acad. Sci. U.S.A. 88:9578-9581).

Identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of expression of a reporter gene, the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The "bait" (*i.e.*, the novel components of the ubiquitin ligase complex of the present invention or derivatives or analogs thereof) and "prey" (proteins to be tested for ability to interact with the bait) proteins are expressed as fusion proteins to a DNA binding domain, and to a transcriptional regulatory domain, respectively, or vice versa. In various specific embodiments, the prey has a complexity of at least about 50, about 100, about 500, about 1,000, about 5,000, about 10,000, or about 50,000; or has a complexity in the range of about 25 to about 100,000, about 100 to about 100,000, about 50,000 to about 100,000, or about 100,000 to about 500,000. For example, the prey population can be one or more nucleic acids encoding mutants of a protein (*e.g.*, as generated by site-directed mutagenesis or another method of making mutations in a nucleotide sequence). Preferably, the prey populations are proteins encoded by DNA, *e.g.*, cDNA or genomic DNA or synthetically-generated DNA. For example, the populations can be expressed from chimeric genes comprising cDNA sequences from an un-characterized sample of a population of cDNA from mRNA.

In a specific embodiment, recombinant biological libraries expressing random peptides can be used as the source of prey nucleic acids.

In general, proteins of the bait and prey populations are provided as fusion (chimeric) proteins (preferably by recombinant expression of a chimeric coding sequence) comprising each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA binding domain. The DNA binding domain can be any DNA binding domain, as long as it specifically recognizes a DNA sequence within a promoter. For example, the DNA binding domain is of a transcriptional activator or inhibitor. For the

other population, the pre-selected sequence is an activator or inhibitor domain of a transcriptional activator or inhibitor, respectively. The regulatory domain alone (not as a fusion to a protein sequence) and the DNA-binding domain alone (not as a fusion to a protein sequence) preferably do not detectably interact (so as to avoid false positives in the assay). The assay system further includes a reporter gene operably linked to a promoter that contains a binding site for the DNA binding domain of the transcriptional activator (or inhibitor). Accordingly, in the present method of the present invention, binding of a ubiquitin ligase fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or inhibitor) which activates (or inhibits) expression of the reporter gene. The activation (or inhibition) of transcription of the reporter gene occurs intracellularly, *e.g.*, in prokaryotic or eukaryotic cells, preferably in cell culture.

The promoter that is operably linked to the reporter gene nucleotide sequence can be a native or non-native promoter of the nucleotide sequence, and the DNA binding site(s) that are recognized by the DNA binding domain portion of the fusion protein can be native to the promoter (if the promoter normally contains such binding site(s)) or non-native to the promoter.

Alternatively, the transcriptional activation binding site of the desired gene(s) can be deleted and replaced with GAL4 binding sites (Bartel et al., 1993, BioTechniques 14:920-924, Chasman et al., 1989, Mol. Cell. Biol. 9:4746-4749). The reporter gene preferably contains the sequence encoding a detectable or selectable marker, the expression of which is regulated by the transcriptional activator, such that the marker is either turned on or off in the cell in response to the presence of a specific interaction. Preferably, the assay is carried out in the absence of background levels of the transcriptional activator (*e.g.*, in a cell that is mutant or otherwise lacking in the transcriptional activator).

The activation domain and DNA binding domain used in the assay can be from a wide variety of transcriptional activator proteins, as long as these transcriptional activators have separable binding and transcriptional activation domains. For example, the GAL4 protein of S. cerevisiae (Ma et al., 1987, Cell 48:847-853), the GCN4 protein of S. cerevisiae (Hope & Struhl, 1986, Cell 46:885-894), the ARD1 protein of S. cerevisiae (Thukral et al., 1989, Mol. Cell. Biol. 9:2360-2369), and the human estrogen receptor (Kumar et al., 1987, Cell 51:941-951), have separable DNA binding and activation domains. The DNA binding domain and activation domain that are employed in the fusion proteins need not be from the same transcriptional activator. In a specific embodiment, a GAL4 or LEXA DNA binding domain is employed. In another specific embodiment, a GAL4 or herpes simplex virus VP16 (Triezenberg et al., 1988, Genes Dev. 2:730-742) activation domain is employed. In a specific embodiment, amino acids 1-147 of GAL4 (Ma

et al., 1987, Cell 48:847-853; Ptashne et al., 1990, Nature 346:329-331) is the DNA binding domain, and amino acids 411-455 of VP16 (Triezenberg et al., 1988, Genes Dev. 2:730-742; Cress et al., 1991, Science 251:87-90) comprise the activation domain.

In a preferred embodiment, the yeast transcription factor GAL4 is reconstituted by protein-protein interaction and the host strain is mutant for GAL4. In another embodiment, the DNA-binding domain is Ace1N and/or the activation domain is Ace1, the DNA binding and activation domains of the Ace1 protein, respectively. Ace1 is a yeast protein that activates transcription from the CUP1 operon in the presence of divalent copper. CUP1 encodes metallothionein, which chelates copper, and the expression of CUP1 protein allows growth in the presence of copper, which is otherwise toxic to the host cells. The reporter gene can also be a CUP1-lacZ fusion that expresses the enzyme beta-galactosidase (detectable by routine chromogenic assay) upon binding of a reconstituted Ace1N transcriptional activator (see Chaudhuri et al., 1995, FEBS Letters 357:221-226). In another specific embodiment, the DNA binding domain of the human estrogen receptor is used, with a reporter gene driven by one or three estrogen receptor response elements (I.e Douarin et al., 1995, Nucl. Acids. Res. 23:876-878). The DNA binding domain and the transcriptional activator/inhibitor domain each preferably has a nuclear localization signal (see Ylikomi et al., 1992, EMBO J. 11:3681-3694, Dingwall and Laskey, 1991, TIBS 16:479-481) functional in the cell in which the fusion proteins are to be expressed.

To facilitate isolation of the encoded proteins, the fusion constructs can further contain sequences encoding affinity tags such as glutathione-S-transferase or maltose-binding protein or an epitope of an available antibody, for affinity purification (*e.g.*, binding to glutathione, maltose, or a particular antibody specific for the epitope, respectively) (Allen et al., 1995, TIBS 20:511-516). In another embodiment, the fusion constructs further comprise bacterial promoter sequences for recombinant production of the fusion protein in bacterial cells.

The host cell in which the interaction assay occurs can be any cell, prokaryotic or eukaryotic, in which transcription of the reporter gene can occur and be detected, including, but not limited to, mammalian (*e.g.*, monkey, mouse, rat, human, bovine), chicken, bacterial, or insect cells, and is preferably a yeast cell. Expression constructs encoding and capable of expressing the binding domain fusion proteins, the transcriptional activation domain fusion proteins, and the reporter gene product(s) are provided within the host cell, by mating of cells containing the expression constructs, or by cell fusion, transformation, electroporation, microinjection, *etc.*

Various vectors and host strains for expression of the two fusion protein populations in yeast are known and can be used (see *e.g.*, U.S. Patent No. 5,1468,614;

Bartel et al., 1993, "Using the two-hybrid system to detect protein-protein interactions" In: Cellular Interactions in Development, Hartley, ed., Practical Approach Series xviii, IRL Press at Oxford University Press, New York, NY, pp. 153-179; Fields and Sternglanz, 1994, Trends In Genetics 10:286-292).

If not already lacking in endogenous reporter gene activity, cells mutant in the reporter gene may be selected by known methods, or the cells can be made mutant in the target reporter gene by known gene-disruption methods prior to introducing the reporter gene (Rothstein, 1983, Meth. Enzymol. 101:202-211).

In a specific embodiment, plasmids encoding the different fusion protein populations can be introduced simultaneously into a single host cell (*e.g.*, a haploid yeast cell) containing one or more reporter genes, by co-transformation, to conduct the assay for protein-protein interactions. Or, preferably, the two fusion protein populations are introduced into a single cell either by mating (*e.g.*, for yeast cells) or cell fusions (*e.g.*, of mammalian cells). In a mating type assay, conjugation of haploid yeast cells of opposite mating type that have been transformed with a binding domain fusion expression construct (preferably a plasmid) and an activation (or inhibitor) domain fusion expression construct (preferably a plasmid), respectively, will deliver both constructs into the same diploid cell. The mating type of a yeast strain may be manipulated by transformation with the HO gene (Herskowitz and Jensen, 1991, Meth. Enzymol. 194:132-146).

In a preferred embodiment, a yeast interaction mating assay is employed using two different types of host cells, strain-type a and alpha of the yeast Saccharomyces cerevisiae. The host cell preferably contains at least two reporter genes, each with one or more binding sites for the DNA-binding domain (*e.g.*, of a transcriptional activator). The activator domain and DNA binding domain are each parts of chimeric proteins formed from the two respective populations of proteins. One strain of host cells, for example the a strain, contains fusions of the library of nucleotide sequences with the DNA-binding domain of a transcriptional activator, such as GAL4. The hybrid proteins expressed in this set of host cells are capable of recognizing the DNA-binding site in the promoter or enhancer region in the reporter gene construct. The second set of yeast host cells, for example, the alpha strain, contains nucleotide sequences encoding fusions of a library of DNA sequences fused to the activation domain of a transcriptional activator.

In another embodiment, the fusion constructs are introduced directly into the yeast chromosome via homologous recombination. The homologous recombination for these purposes is mediated through yeast sequences that are not essential for vegetative growth of yeast, *e.g.*, the MER2, MER1, ZIP1, REC102, or ME14 gene.

Bacteriophage vectors can also be used to express the DNA binding domain and/or activation domain fusion proteins. Libraries can generally be prepared faster and more easily from bacteriophage vectors than from plasmid vectors.

In a specific embodiment, the present invention provides a method of detecting one or more protein-protein interactions comprising (a) recombinantly expressing a novel ubiquitin ligase component of the present invention or a derivative or analog thereof in a first population of yeast cells being of a first mating type and comprising a first fusion protein containing the sequence of a novel ubiquitin ligase component of the present invention and a DNA binding domain, wherein said first population of yeast cells contains a first nucleotide sequence operably linked to a promoter driven by one or more DNA binding sites recognized by said DNA binding domain such that an interaction of said first fusion protein with a second fusion protein, said second fusion protein comprising a transcriptional activation domain, results in increased transcription of said first nucleotide sequence; (b) negatively selecting to eliminate those yeast cells in said first population in which said increased transcription of said first nucleotide sequence occurs in the absence of said second fusion protein; (c) recombinantly expressing in a second population of yeast cells of a second mating type different from said first mating type, a plurality of said second fusion proteins, each second fusion protein comprising a sequence of a fragment, derivative or analog of a protein and an activation domain of a transcriptional activator, in which the activation domain is the same in each said second fusion protein; (d) mating said first population of yeast cells with said second population of yeast cells to form a third population of diploid yeast cells, wherein said third population of diploid yeast cells contains a second nucleotide sequence operably linked to a promoter driven by a DNA binding site recognized by said DNA binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of said second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different; and (e) detecting said increased transcription of said first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

5.4.2 ASSAYS TO IDENTIFY F-BOX PROTEIN INTERACTIONS WITH KNOWN PROTEINS INCLUDING POTENTIAL SUBSTRATES

The cellular abundance of cell-cycle regulatory proteins, such as members of the cyclin family or the Cki inhibitory proteins, is regulated by the ubiquitin pathway. The enzymes responsible for the ubiquitination of mammalian cell cycle regulation are not known. In yeast, SCF complexes represent the ubiquitin ligases for cell cycle regulators.

The F-box component of the ubiquitin ligase complexes, such as the novel F-box proteins of the invention, determines the specificity of the target of the ubiquitin ligase complex. The invention therefore provides assays to screen known molecules for specific binding to F-box protein nucleic acids, proteins, or derivatives under conditions conducive to binding, and then molecules that specifically bind to the FBP protein are identified.

In a specific embodiment, the invention provides a method for studying the interaction between the F-box protein FBP1 and the Cul1/Skp1 complex, and its role in regulating the stability of β-catenin. Protein-protein interactions can be probed in vivo and in vitro using antibodies specific to these proteins, as described in detail in the experiments in Section 8.

In another specific embodiment, the invention provides for a method for detecting the interaction between the F-box protein Skp2 and E2F-1, a transcription factor involved in cell cycle progression. Insect cells can be infected with baculoviruses co-expressing Skp2 and E2F-1, and cell extracts can be prepared and analyzed for protein-protein interactions. As described in detail in Section 7, this assay has been used successfully to identify potential targets, such as E2F, for known F-box proteins, such as Skp2. This assay can be used to identify other Skp2 targets, as well as targets for novel F-box proteins.

In another specific embodiment, methods for detecting the interaction between Skp2 and p27, a cell cycle regulated cyclin-dependent kinase (Cdk) inhibitor, are provided. The interaction between Skp2 and p27 may be targeted to identify modulators of Skp2 activity, including its interaction with cell cycle regulators, such as p27. The ubiquitination of Skp2-specific substrates, such as p27 may be used as a means of measuring the ability of a test compound to modulate Skp2 activity. In another embodiment of the screening assays of the present invention, immunodepletion assays, as described in Section 9, can be used to identify modulators of the Skp2/p27 interaction. In particular, Section 9 describes a method for detection of ubiquitination activity in vitro using p27 as a substrate, which can also be used to identify modulators of the Skp2-dependent ubiquitination of p27. In another embodiment of the screening assays of the present invention, antisense oligonucleotides, as described in Section 5.7.1, can be used as inhibitors of the Skp2 activity. Such identified modulators of p27 ubiquitination/degradation and of the Skp2/p27 interaction can be useful in anti-cancer therapies.

The invention further provides methods for screening ubiquitin ligase complexes having novel F-box proteins (or fragments thereof) as one of their components for ubiquitin ligase activity using known cell-cycle regulatory molecules as potential

substrates for ubiquitination. For example, cells engineered to express FBP nucleic acids can be used to recombinantly produce FBP proteins either wild-type or dominant negative mutants in cells that also express a putative ubiquitin-ligase substrate molecule. Such candidates for substrates of the novel FBP of the present invention include, but are not limited to, such potential substrates as IKBα, β-catenin, myc, E2F-1, p27, p21, cyclin A, cyclin B, cycD1, cyclin E and p53. Then the extracts can be used to test the association of F-box proteins with their substrates, (by Western blot immunoassays) and whether the presence of the FBP increases or decreases the level of the potential substrates.

5.5 ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS THAT MODULATE THE ACTIVITY OF F-BOX PROTEINS

The present invention relates to in vitro and in vivo assay systems described in the subsections below, which can be used to identify compounds or compositions that modulate the interaction of known FBPs with novel substrates and novel components of the ubiquitin ligase complex. The screening assays of the present invention may also be used to identify compounds or compositions that modulate the interaction of novel FBPs with their identified substrates and components of the ubiquitin ligase complex.

Methods to screen potential agents for their ability to disrupt or moderate FBP expression and activity can be designed based on the Applicants' discovery of novel FBPs and their interaction with other components of the ubiquitin ligase complex as well as its known and potential substrates. For example, candidate compounds can be screened for their ability to modulate the interaction of an FBP and Skp1, or the specific interactions of Skp2 with E2F-1, Skp2 with p27, or the FBP1/Cul1/Skp1 complex with β-catenin. In principle, many methods known to those of skill in the art, can be readily adapted in designed the assays of the present invention.

The screening assays of the present invention also encompass high-throughput screens and assays to identify modulators of FBP expression and activity. In accordance with this embodiment, the systems described below may be formulated into kits. To this end, cells expressing FBP and components of the ubiquitination ligase complex and the ubiquitination pathway, or cell lysates, thereof can be packaged in a variety of containers, *e.g.*, vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the kit; *e.g.*, positive control samples, negative control samples, buffers, cell culture media, *etc.*

The invention provides screening methodologies useful in the identification of proteins and other compounds which bind to, or otherwise directly interact with, the FBP genes and their gene products. Screening methodologies are well known in the art (see *e.g.*,

PCT International Publication No. WO 96/34099, published October 31, 1996, which is incorporated by reference herein in its entirety). The proteins and compounds include endogenous cellular components which interact with the identified genes and proteins in vivo and which, therefore, may provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic, and otherwise exogenous compounds which may have binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one series of embodiments, cell lysates or tissue homogenates may be screened for proteins or other compounds which bind to one of the normal or mutant FBP genes and FBP proteins.

Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (*e.g.*, libraries of small molecules or peptides), may be screened for binding capacity. All of these methods comprise the step of mixing an FBP protein or fragment with test compounds, allowing time for any binding to occur, and assaying for any bound complexes. All such methods are enabled by the present disclosure of substantially pure FBP proteins, substantially pure functional domain fragments, fusion proteins, antibodies, and methods of making and using the same.

5.5.1 ASSAYS FOR F-BOX PROTEIN AGONISTS AND ANTAGONISTS

FBP nucleic acids, F-box proteins, and derivatives can be used in screening assays to detect molecules that specifically bind to FBP nucleic acids, proteins, or derivatives and thus have potential use as agonists or antagonists of FBPs, in particular, molecules that thus affect cell proliferation. In a preferred embodiment, such assays are performed to screen for molecules with potential utility as anti-cancer drugs or lead compounds for drug development. The invention thus provides assays to detect molecules that specifically bind to FBP nucleic acids, proteins, or derivatives. For example, recombinant cells expressing FBP nucleic acids can be used to recombinantly produce FBP proteins in these assays, to screen for molecules that bind to an FBP protein. Similar methods can be used to screen for molecules that bind to FBP derivatives or nucleic acids. Methods that can be used to carry out the foregoing are commonly known in the art. The assays of the present invention may be first optimized on a small scale (*i.e.*, in test tubes), and then scaled up for high-throughput assays. The screening assays of the present may be performed in vitro, *i.e.* in test tubes, using purified components or cell lysates. The screening assays of the present invention may also be carried out in intact cells in culture and in animal models. In accordance with the present invention, test compounds which are shown to modulate the activity of the FBP as described herein in vitro, will further be assayed in vivo, including cultured cells and animal models to determine if the test

compound has the similar effects in vivo and to determine the effects of the test compound on cell cycle progression, the accumulation or degradation of positive and negative regulators, cellular proliferation *etc.*

In accordance with the present invention, screening assays may be designed to detect molecules which act as agonists or antagonists of the activity of the novel F-box proteins. In accordance with this aspect of the invention, the test compound may be added to an assay system to measure its effect on the activity of the novel FBP, *i.e.*, ubiquitination of its substrates, interaction with other components of the ubiquitin ligase complex, *etc.* These assays should be conducted both in the presence and absence of the test compound.

In accordance with the present invention, ubiquitination activity of a novel FBP in the presence or absence of a test compound can be measured in vitro using purified components of the ubiquitination pathway or may be measured using crude cellular extracts obtained from tissue culture cells or tissue samples. In another embodiment of the aspect of the present invention the screening may be performed by adding the test agent to in vitro translation systems such as a rabbit reticulocyte lysate (RRL) system and then proceeding with the established analysis. As another alternative, purified or partially purified components which have been determined to interact with one another by the methods described above can be placed under conditions in which the interaction between them would normally occur, with and without the addition of the test agent, and the procedures previously established to analyze the interaction can be used to assess the impact of the test agent. In this approach, the purified or partially purified components may be prepared by fractionation of extracts of cells expressing the components of the ubiquitin ligase complex and pathway, or they may be obtained by expression of cloned genes or cDNAs or fragments thereof, optionally followed by purification of the expressed material.

Within the broad category of in vitro selection methods, several types of method are likely to be particularly convenient and/or useful for screening test agents. These include but are not limited to methods which measure a binding interaction between two or more components of the ubiquitin ligase complex or interaction with the target substrate, methods which measure the activity of an enzyme which is one of the interacting components, and methods which measure the activity or expression of "reporter" protein, that is, an enzyme or other detectable or selectable protein, which has been placed under the control of one of the components.

Binding interactions between two or more components can be measured in a variety of ways. One approach is to label one of the components with an easily detectable label, place it together with the other component(s) in conditions under which they would normally interact, perform a separation step which separates bound labeled component from

unbound labeled component, and then measure the amount of bound component. The effect of a test agent included in the binding reaction can be determined by comparing the amount of labeled component which binds in the presence of this agent to the amount which binds in its absence.

In another embodiment, screening can be carried out by contacting the library members with an FBP protein (or nucleic acid or derivative) immobilized on a solid phase and harvesting those library members that bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley & Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited hereinabove.

In another embodiment, the two-hybrid system for selecting interacting proteins or peptides in yeast (Fields & Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to an FBP protein or derivative.

Alternatively, test methods may rely on measurements of enzyme activity, such as ubiquitination of the target substrate. Once a substrate of a novel FBP is identified or a novel putative substrate of a known FBP is identified, such as the novel substrates of Skp2, E2F and p27, these components may be used in assays to determine the effect of a test compound on the ubiquitin ligase activity of the ubiquitin ligase complex.

In one embodiment, the screening assays may be conducted with a purified system in the presence and absence of test compound. Purified substrate is incubated together with purified ubiquitin ligase complex, ubiquitin conjugating enzymes, ubiquitin activating enzymes and ubiquitin in the presence or in the absence of test compound. Ubiquitination of the substrate is analyzed by immunoassay (see Pagano et al., 1995, Science 269:682-685). Briefly, ubiquitination of the substrate can be performed in vitro in reactions containing 50-200ng of proteins in 50mM Tris pH 7.5, 5mM MgCl2, 2mM ATPγ-S, 0.1 mM DTT and 5μM of biotinylated ubiquitin. Total reactions (30μl) can be incubated at 25°C for up to 3 hours in the presence or absence of test compound and then loaded on an 8% SDS gel or a 4-20% gradient gel for analysis. The gels are run and proteins are electrophoretically transferred to nitrocellulose. Ubiquitination of the substrate can be detected by immunoblotting. Ubiquitinated substrates can be visualized using Extravidin-HRP (Sigma), or by using a substrate-specific antibody, and the ECL detection system (NEN).

In another embodiment, ubiquitination of the substrate may be assayed in intact cells in culture or in animal models in the presence and absence of the test compound.

For example, the test compound may be administered directly to an animal model or to crude extracts obtained from animal tissue samples to measure ubiquitination of the substrate in the presence and absence of the test compounds. For these assays, host cells to which the test compound is added may be genetically engineered to express the FBP components of the ubiquitin ligase pathway and the target substrate, the expression of which may be transient, induced or constitutive, or stable. For the purposes of the screening methods of the present invention, a wide variety of host cells may be used including, but not limited to, tissue culture cells, mammalian cells, yeast cells, and bacteria. Each cell type has its own set of advantages and drawbacks. Mammalian cells such as primary cultures of human tissue cells may be a preferred cell type in which to carry out the assays of the present invention, however these cell types are sometimes difficult to cultivate. Bacteria and yeast are relatively easy to cultivate but process proteins differently than mammalian cells. This ubiquitination assay may be conducted as follows: first, the extracts are prepared from human or animal tissue. To prepare animal tissue samples preserving ubiquitinating enzymes, 1 g of tissue can be sectioned and homogenized at 15,000 r.p.m. with a Brinkmann Polytron homogenizer (PT 3000, Westbury, NY) in 1 ml of ice-cold double-distilled water. The sample is frozen and thawed 3 times. The lysate is spun down at 15,000 r.p.m. in a Beckman JA-20.1 rotor (Beckman Instruments, Palo Alto, CA) for 45 min at 4°C. The supernatant is retrieved and frozen at -80°C. This method of preparation of total extract preserves ubiquitinating enzymes (Loda et al. 1997, Nature Medicine 3:231-234, incorporated by reference herein in its entirety).

Purified recombinant substrate is added to the assay system and incubated at 37°C for different times in 30 µl of ubiquitination mix containing 100 µg of protein tissue homogenates, 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, and 1 mM DTT, 2 mM ATP, 10 mM creatine phosphokinase, 10 mM creatine phosphate and 5 µM biotinylated ubiquitin. The substrate is then re-purified with antibodies or affinity chromatography. Ubiquitination of the substrate is measured by immunoassays with either antibodies specific to the substrates or with Extravidin-HRP.

In addition, Drosophila can be used as a model system in order to detect genes that phenotypically interact with FBP. For example, overexpression of FBP in Drosophila eye leads to a smaller and rougher eye. Mutagenesis of the fly genome can be performed, followed by selecting flies in which the mutagenesis has resulted in suppression or enhancement of the small rough eye phenotype; the mutated genes in such flies are likely to encode proteins that interact/bind with FBP. Active compounds identified with methods described above will be tested in cultured cells and/or animal models to test the effect of

blocking in vivo FBP activity (*e.g.* effects on cell proliferation, accumulation of substrates, *etc.*).

In various other embodiments, screening the can be accomplished by one of many commonly known methods. See, *e.g.*, the following references, which disclose screening of peptide libraries: Parmley & Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott & Smith, 1990, Science 249:386-390; Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-565; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner et al.; Rebar & Pabo, 1993, Science 263:671-673; and PCT Publication No. WO 94/18318.

Compounds, peptides, and small molecules can be used in screening assays to identify candidate agonists and antagonists. In one embodiment, peptide libraries may be used to screen for agonists or antagonists of the FBP of the present invention diversity libraries, such as random or combinatorial peptide or non-peptide libraries can be screened for molecules that specifically bind to FBP. Many libraries are known in the art that can be used, *e.g.*, chemically synthesized libraries, recombinant (*e.g.*, phage display libraries), and in vitro translation-based libraries.

Examples of chemically synthesized libraries are described in Fodor et al., 1991, Science 251:767-773; Houghten et al., 1991, Nature 354:84-86; Lam et al., 1991, Nature 354:82-84; Medynski, 1994, Bio/Technology 12:709-710; Gallop et al., 1994, J. Medicinal Chemistry 37(9):1233-1251; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten et al., 1992, Biotechniques 13:412; Jayawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

Examples of phage display libraries are described in Scott & Smith, 1990, Science 249:386-390; Devlin et al., 1990, Science, 249:404-406; Christian, et al., 1992, J. Mol. Biol. 227:711-718; Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay et al., 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated August 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated April 18, 1991; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

By way of examples of non-peptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

5.5.2 ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS THAT MODULATE THE INTERACTION OF F-box PROTEINS WITH OTHER PROTEINS

Once a substrate or interacting protein is identified, as described in detail in Section 5.4, then one can assay for modulators of the F-box protein interaction with such a protein. The present invention provides for methods of detecting agonists and antagonists of such interactions.

In one embodiment, the invention encompasses methods to identify modulators, such as inhibitors or agonists, of the interaction between the F-box protein Skp2 and E2F-1, identified in Section 7 and Figure 10. Such methods comprise both in vivo and in vitro assays for modulator activity. For example, in an in vivo assay, insect cells can be co-infected with baculoviruses co-expressing Skp2 and E2F-1 as well as potential modulators of the Skp2/E2F-1 interaction. The screening methods of the present invention encompass in vitro assays which measure the ability of a test compound to inhibit the enzymatic activity of Skp2 as described above in Section 5.5.1. Cell extracts can be prepared and analyzed for protein-protein interactions by gel electrophoresis and detected by immunoblotting, as described in detail in Section 7 and presented in Figure 10. Alternatively, an in vitro protein-protein interaction assay can be used. Recombinant purified Skp2, E2F-1, and putative agonist or antagonist molecules can be incubated together, under conditions that allow binding to occur, such as 37 C for 30 minutes. Protein-protein complex formation can be detected by gel analysis, such as those described herein in Section 7. This assay can be used to identify modulators of interactions of known FBP, such as Skp2 with novel substrates.

In another embodiment, the invention provides for a method for identification of modulators of F-box protein/Skp1 interaction. Such agonist and antagonists can be identified in vivo or in vitro. For example, in an in vitro assay to identify modulators of F-box protein/Skp1 interactions, purified Skp1 and the novel FBP can be incubated together, under conditions that allow binding occur, such as 37C for 30 minutes. In a parallel reaction, a potential agonist or antagonist, as described above in Section 5.5.1, is added either before or during the box protein/Skp1 incubation. Protein-protein

interactions can be detected by gel analysis, such as those described herein in Section 7. Modulators of FBP activities and interactions with other proteins can be used as therapeutics using the methods described herein, in Section 5.7.

These assays may be carried out utilizing any of the screening methods described herein, including the following in vitro assay. The screening can be performed by adding the test agent to intact cells which express components of the ubiquitin pathway, and then examining the component of interest by whatever procedure has been established. Alternatively, the screening can be performed by adding the test agent to in vitro translation reactions and then proceeding with the established analysis. As another alternative, purified or partially purified components which have been determined to interact with one another by the methods described above can be placed under conditions in which the interaction between them would normally occur, with and without the addition of the test agent, and the procedures previously established to analyze the interaction can be used to assess the impact of the test agent. In this approach, the purified or partially purified components may be prepared by fractionation of extracts of cells expressing the components of the ubiquitin ligase complex and pathway, or they may be obtained by expression of cloned genes or cDNAs or fragments thereof, optionally followed by purification of the expressed material.

Within the broad category of in vitro selection methods, several types of method are likely to be particularly convenient and/or useful for screening test agents. These include but are not limited to methods which measure a binding interaction between two or more components of the ubiquitin ligase complex or interaction with the target substrate, methods which measure the activity of an enzyme which is one of the interacting components, and methods which measure the activity or expression of "reporter" protein, that is, an enzyme or other detectable or selectable protein, which has been placed under the control of one of the components.

Binding interactions between two or more components can be measured in a variety of ways. One approach is to label one of the components with an easily detectable label, place it together with the other component(s) in conditions under which they would normally interact, perform a separation step which separates bound labeled component from unbound labeled component, and then measure the amount of bound component. The effect of a test agent included in the binding reaction can be determined by comparing the amount of labeled component which binds in the presence of this agent to the amount which binds in its absence.

The separation step in this type of procedure can be accomplished in various ways. In one approach, (one of) the binding partner(s) for the labeled component can be immobilized on a solid phase prior to the binding reaction, and unbound labeled component

can be removed after the binding reaction by washing the solid phase. Attachment of the binding partner to the solid phase can be accomplished in various ways known to those skilled in the art, including but not limited to chemical cross-linking, non-specific adhesion to a plastic surface, interaction with an antibody attached to the solid phase, interaction between a ligand attached to the binding partner (such as biotin) and a ligand-binding protein (such as avidin or streptavidin) attached to the solid phase, and so on.

Alternatively, the separation step can be accomplished after the labeled component had been allowed to interact with its binding partner(s) in solution. If the size differences between the labeled component and its binding partner(s) permit such a separation, the separation can be achieved by passing the products of the binding reaction through an ultrafilter whose pores allow passage of unbound labeled component but not of its binding partner(s) or of labeled component bound to its partner(s). Separation can also be achieved using any reagent capable of capturing a binding partner of the labeled component from solution, such as an antibody against the binding partner, a ligand-binding protein which can interact with a ligand previously attached to the binding partner, and so on.

5.6 METHODS AND COMPOSITIONS FOR DIAGNOSTIC USE OF F-BOX PROTEINS, DERIVATIVES, AND MODULATORS

Cell cycle regulators are the products of oncogenes (cyclins, β-catenin, *etc.*), or tumor suppressor genes (ckis, p53, *etc.*) The FBPs, part of ubiquitin ligase complexes, might therefore be products of oncogenes or tumor suppressor genes, depending on which cell cycle regulatory proteins for which they regulate cellular abundance.

FBP proteins, analogues, derivatives, and subsequences thereof, FBP nucleic acids (and sequences complementary thereto), anti-FBP antibodies, have uses in diagnostics. The FBP and FBP nucleic acids can be used in assays to detect, prognose, or diagnose proliferative or differentiative disorders, including tumorigenesis, carcinomas, adenomas *etc.* The novel FBP nucleic acids of the present invention are located at chromosome sites associated with karyotypic abnormalities and loss of heterozygosity. The FBP1 nucleic acid of the present invention is mapped and localized to chromosome position 10q24, the loss of which has been demonstrated in 10 % of human prostate tumors and small cell lung carcinomas (SCLC), suggesting the presence of a tumor suppressor gene at this location. In addition, up to 7% of childhood acute T-cell leukemia is accompanied by a translocation involving 10q24 as a breakpoint, either t(10;14)(q24;q11) or t(7;10)(q35;q24). 9q34 region (where FBP2 is located) has been shown to be a site of loss of heterozygosity (LOH) in human ovarian and bladder cancers. The FBP2 nucleic acid of the present

invention is mapped and localized to chromosome position 9q34 which has been shown to be a site of loss of heterozygosity (LOH) in human ovarian and bladder cancers. The FBP3 nucleic acid of the present invention is mapped and localized to chromosome position 13q22, a region known to contain a putative tumor suppressor gene with loss of heterozygosity in approx. 75 % of human SCLC. The FBP4 nucleic acid of the present invention is mapped and localized to chromosome position 5p12, a region shown to be a site of karyotypic abnormalities in a variety of tumors, including human breast cancer and nasopharyngeal carcinomas. The FBP5 nucleic acid of the present invention is mapped and localized to chromosome position 6q25-26, a region shown to be a site of loss of heterozygosity in human ovarian, breast and gastric cancers hepatocarcinomas, Burkitt's lymphomas, gliomas, and parathyroid adenomas. The FBP7 nucleic acid of the present invention is mapped and localized to chromosome position 15q15 a region which contains a tumor suppressor gene associated with progression to a metastatic stage in breast and colon cancers and a loss of heterozygosity in parathyroid adenomas.

The molecules of the present invention can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting FBP expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-FBP antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, can be used to detect aberrant FBP localization or aberrant (*e.g.*, low or absent) levels of FBP. In a specific embodiment, antibody to FBP can be used to assay a patient tissue or serum sample for the presence of FBP where an aberrant level of FBP is an indication of a diseased condition. By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, immunohisto-chemistry radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

FBP genes and related nucleic acid sequences and subsequences, including complementary sequences, can also be used in hybridization assays. FBP nucleic acid

sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in FBP expression and/or activity as described supra. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to FBP DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

In specific embodiments, diseases and disorders involving overproliferation of cells can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting decreased levels of FBP protein, FBP RNA, or FBP functional activity (*e.g.*, ubiquitin ligase target binding activity, F-box domain binding activity, ubiquitin ligase activity *etc.*), or by detecting mutations in FBP RNA, DNA or FBP protein (*e.g.*, translocations in FBP nucleic acids, truncations in the FBP gene or protein, changes in nucleotide or amino acid sequence relative to wild-type FBP) that cause decreased expression or activity of FBP. Such diseases and disorders include but are not limited to those described in Section 5.7.3. By way of example, levels of FBP protein can be detected by immunoassay, levels of FBP RNA can be detected by hybridization assays (*e.g.*, Northern blots, in situ-hybridization), FBP activity can be assayed by measuring ubiquitin ligase activity in E3 ubiquitin ligase complexes formed in vivo or in vitro, F-box domain binding activity can be assayed by measuring binding to Skp1 protein by binding assays commonly known in the art, translocations, deletions and point mutations in FBP nucleic acids can be detected by Southern blotting, FISH, RFLP analysis, SSCP, PCR using primers that preferably generate a fragment spanning at least most of the FBP gene, sequencing of FBP genomic DNA or cDNA obtained from the patient, *etc.*

In a preferred embodiment, levels of FBP mRNA or protein in a patient sample are detected or measured, in which decreased levels indicate that the subject has, or has a predisposition to developing, a malignancy or hyperproliferative disorder; in which the decreased levels are relative to the levels present in an analogous sample from a portion of the body or from a subject not having the malignancy or hyperproliferative disorder, as the case may be.

In another specific embodiment, diseases and disorders involving a deficiency in cell proliferation or in which cell proliferation is desirable for treatment, are diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting increased levels of FBP protein, FBP RNA, or FBP functional activity (*e.g.*, ubiquitin ligase activity, Skp1 binding activity, *etc.*), or by

detecting mutations in FBP RNA, DNA or protein (*e.g.*, translocations in FBP nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type FBP) that cause increased expression or activity of FBP. Such diseases and disorders include but are not limited to those described in Section 5.7.3. By way of example, levels of FBP protein, levels of FBP RNA, ubiquitin ligase activity, FBP binding activity, and the presence of translocations or point mutations can be determined as described above.

In a specific embodiment, levels of FBP mRNA or protein in a patient sample are detected or measured, in which increased levels indicate that the subject has, or has a predisposition to developing, a growth deficiency or degenerative or hypoproliferative disorder; in which the increased levels are relative to the levels present in an analogous sample from a portion of the body or from a subject not having the growth deficiency, degenerative, or hypoproliferative disorder, as the case may be.

Kits for diagnostic use are also provided, that comprise in one or more containers an anti-FBP antibody, and, optionally, a labeled binding partner to the antibody. Alternatively, the anti-FBP antibody can be labeled (with a detectable marker, *e.g.*, a chemiluminescent, enzymatic, fluorescent, or radioactive moiety). A kit is also provided that comprises in one or more containers a nucleic acid probe capable of hybridizing to FBP RNA. In a specific embodiment, a kit can comprise in one or more containers a pair of primers (*e.g.*, each in the size range of 6-30 nucleotides) that are capable of priming amplification [*e.g.*, by polymerase chain reaction (see *e.g.*, Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Q replicase, cyclic probe reaction, or other methods known in the art] under appropriate reaction conditions of at least a portion of a FBP nucleic acid. A kit can optionally further comprise in a container a predetermined amount of a purified FBP protein or nucleic acid, *e.g.*, for use as a standard or control.

5.7 METHODS AND COMPOSITIONS FOR THERAPEUTIC USE OF F-box PROTEINS, DERIVATIVES, AND MODULATORS

Described below are methods and compositions for the use of F-box proteins in the treatment of proliferative disorders and oncogenic disease symptoms may be ameliorated by compounds that activate or enhance FBP activity, and whereby proliferative disorders and cancer may be ameliorated.

In certain instances, compounds and methods that increase or enhance the activity of an FBP can be used to treat proliferative and oncogenic disease symptoms. Such a case may involve, for example, a proliferative disorder that is brought about, at least in

part, by a reduced level of FBP gene expression, or an aberrant level of an FBP gene product's activity. For example, decreased activity or under-expression of an FBP component of a ubiquitin ligase complex whose substrate is a positive cell-cycle regulator, such as a member of the Cyclin family, will result in increased cell proliferation. As such, an increase in the level of gene expression and/or the activity of such FBP gene products would bring about the amelioration of proliferative disease symptoms.

In another instance, compounds that increase or enhance the activity of an FBP can be used to treat proliferative and oncogenic disease symptoms resulting from defects in the expression or activity of other genes and gene products involved in cell cycle control, such as FBP substrate molecules. For example, an increase in the expression or activity of a positive cell-cycle positive molecule, such as a member of the Cyclin family, may result in its over-activity and thereby lead to increased cell proliferation. Compounds that increase the expression or activity of the FBP component of a ubiquitin ligase complex whose substrate is such a cell-cycle positive regulator will lead to ubiquitination of the defective molecule, and thereby result in an increase in its degradation. Disease symptoms resulting from such a defect may be ameliorated by compounds that compensate the disorder by increased FBP activity. Techniques for increasing FBP gene expression levels or gene product activity levels are discussed in Section 5.7, below.

Alternatively, compounds and methods that reduce or inactivate FBP activity may be used therapeutically to ameliorate proliferative and oncogenic disease symptoms. For example, a proliferative disorder may be caused, at least in part, by a defective FBP gene or gene product that leads to its overactivity. Where such a defective gene product is a component of a ubiquitin ligase complex whose target is a cell-cycle inhibitor molecule, such as a Cki, an overactive FBP will lead to a decrease in the level of cell-cycle molecule and therefore an increase in cell proliferation. In such an instance, compounds and methods that reduce or inactivate FBP function may be used to treat the disease symptoms.

In another instance, compounds and methods that reduce the activity of an FBP can be used to treat disorders resulting from defects in the expression or activity of other genes and gene products involved in cell cycle control, such as FBP substrate molecules. For example, a defect in the expression or activity of a cell-cycle negative regulatory molecule, such as a Cki, may lead to its under-activity and thereby result in increased cell proliferation. Reduction in the level and/or activity of an FBP component whose substrate was such molecule would decrease the ubiquitination and thereby increase the level of such a defective molecule. Therefore, compounds and methods aimed at reducing the expression and/or activity of such FBP molecules could thereby be used in the treatment of disease symptoms by compensating for the defective gene or gene product.

Techniques for the reduction of target gene expression levels or target gene product activity levels are discussed in Section 5.7 below.

5.7.1 THERAPEUTIC USE OF INHIBITORY ANTISENSE, RIBOZYME AND TRIPLE HELIX MOLECULES AND IDENTIFIED AGONISTS AND ANTAGONISTS

In another embodiment, symptoms of certain FBP disorders, such as such as proliferative or differentiative disorders causing tumorigenesis or cancer, may be ameliorated by decreasing the level of FBP gene expression and/or FBP gene product activity by using FBP gene sequences in conjunction with well-known antisense, gene "knock-out" ribozyme and/or triple helix methods to decrease the level of FBP gene expression. Among the compounds that may exhibit the ability to modulate the activity, expression or synthesis of the FBP gene, including the ability to ameliorate the symptoms of an FBP disorder, such as cancer, are antisense, ribozyme, and triple helix molecules. Such molecules may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant target gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art. For example, antisense targeting SKP2 mRNA stabilize the Skp2-substrate p27, as described in Section X (Figure X).

Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides that are complementary to a target gene mRNA. The antisense oligonucleotides will bind to the complementary target gene mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required.

A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In one embodiment, oligonucleotides complementary to non-coding regions of the FBP gene could be used in an antisense approach to inhibit translation of endogenous FBP mRNA. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific

aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

In an embodiment of the present invention, oligonucleotides complementary to the nucleic acids encoding the F-box motif as indicated in Figures 2 and 4-9.

Regardless of the choice of target sequence, it is preferred that in vitro studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, *etc.* The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger, et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86, 6553-6556; Lemaitre, et al., 1987, Proc. Natl. Acad. Sci. 84, 648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6, 958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5, 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, *etc.*

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-

methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5 -methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v). wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate (S-ODNs), a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an -anomeric oligonucleotide. An -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual -units, the strands run parallel to each other (Gautier, et al., 1987, Nucl. Acids Res. 15, 6625-6641). The oligonucleotide is a 2 -0-methylribonucleotide (Inoue, et al., 1987, Nucl. Acids Res. 15, 6131-6148), or a chimeric RNA-DNA analogue (Inoue, et al., 1987, FEBS Lett. 215, 327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, *etc.*). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein, et al. (1988, Nucl. Acids Res. 16, 3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin, et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85, 7448-7451), *etc.*

While antisense nucleotides complementary to the target gene coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

In one embodiment of the present invention, gene expression downregulation is achieved because specific target mRNAs are digested by RNAse H after they have hybridized with the antisense phosphorothioate oligonucleotides (S-ODNs). Since no rules exist to predict which antisense S-ODNs will be more successful, the best strategy is completely empirical and consists of trying several antisense S-ODNs. Antisense phosphorothioate oligonucleotides (S-ODNs) will be designed to target specific regions of

mRNAs of interest. Control S-ODNs consisting of scrambled sequences of the antisense S-ODNs will also be designed to assure identical nucleotide content and minimize differences potentially attributable to nucleic acid content. All S-ODNs will be synthesized by Oligos *Etc.* (Wilsonville, OR). In order to test the effectiveness of the antisense molecules when applied to cells in culture, such as assays for research purposes or ex vivo gene therapy protocols, cells will be grown to 60-80% confluence on 100 mm tissue culture plates, rinsed with PBS and overlaid with lipofection mix consisting of 8 ml Opti-MEM, 52.8 l Lipofectin, and a final concentration of 200 nM S-ODNs. Lipofections will be carried out using Lipofectin Reagent and Opti-MEM (Gibco BRL). Cells will be incubated in the presence of the lipofection mix for 5 hours. Following incubation the medium will be replaced with complete DMEM. Cells will be harvested at different time points post-lipofection and protein levels will be analyzed by Western blot.

Antisense molecules should be targeted to cells that express the target gene, either directly to the subject in vivo or to cells in culture, such as in ex vivo gene therapy protocols. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous target gene transcripts and thereby prevent translation of the target gene mRNA. For example, a vector can be introduced *e.g.*, such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290, 304-310), the promoter contained in the 3 long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980,

Cell 22, 787-797), the herpes thymidine kinase promoter (Wagner, et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78, 1441-1445), the regulatory sequences of the metallothioncin gene (Brinster, et al., 1982, Nature 296, 39-42), *etc.* Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral vectors can be used that selectively infect the desired tissue, in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of target gene mRNA and, therefore, expression of target gene product (see, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver, et al., 1990, Science 247, 1222-1225). In an embodiment of the present invention, oligonucleotides which hybridize to the FBP gene are designed to be complementary to the nucleic acids encoding the F-box motif as indicated in Figures 2 and 4-9.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4, 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff & Gerlach, 1988, Nature, 334, 585-591, which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target gene mRNA, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in Tetrahymena

thermophila (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224, 574-578; Zaug and Cech, 1986, Science, 231, 470-475; Zaug, et al., 1986, Nature, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been & Cech, 1986, Cell, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, *etc.*) and should be delivered to cells that express the target gene in vivo. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies, et al., 1985, Nature 317, 230-234; Thomas & Capecchi, 1987, Cell 51, 503-512; Thompson, et al., 1989, Cell 5, 313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene in vivo. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas & Capecchi, 1987 and Thompson, 1989, supra). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors.

Alternatively, endogenous target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (*i.e.*, the target gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target gene in target cells in the body. (See generally,

Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC+ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles that the possibility may arise wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity may, be introduced into cells via gene therapy methods such as those described, below, in Section 5.7.2 that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing

oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

5.7.2 GENE REPLACEMENT THERAPY

With respect to an increase in the level of normal FBP gene expression and/or FBP gene product activity, FBP gene nucleic acid sequences, described, above, in Section 5.1 can, for example, be utilized for the treatment of proliferative disorders such as cancer. Such treatment can be administered, for example, in the form of gene replacement therapy. Specifically, one or more copies of a normal FBP gene or a portion of the FBP gene that directs the production of an FBP gene product exhibiting normal FBP gene function, may be inserted into the appropriate cells within a patient, using vectors that include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

For FBP genes that are expressed in all tissues or are preferentially expressed, such as FBP1 gene is expressed preferably in the brain, such gene replacement therapy techniques should be capable delivering FBP gene sequences to these cell types within patients. Thus, in one embodiment, techniques that are well known to those of skill in the art (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988) can be used to enable FBP gene sequences to cross the blood-brain barrier readily and to deliver the sequences to cells in the brain. With respect to delivery that is capable of crossing the blood-brain barrier, viral vectors such as, for example, those described above, are preferable.

In another embodiment, techniques for delivery involve direct administration of such FBP gene sequences to the site of the cells in which the FBP gene sequences are to be expressed.

Additional methods that may be utilized to increase the overall level of FBP gene expression and/or FBP gene product activity include the introduction of appropriate FBP-expressing cells, preferably autologous cells, into a patient at positions and in numbers that are sufficient to ameliorate the symptoms of an FBP disorder. Such cells may be either recombinant or non-recombinant.

Among the cells that can be administered to increase the overall level of FBP gene expression in a patient are cells that normally express the FBP gene.

Alternatively, cells, preferably autologous cells, can be engineered to express FBP gene sequences, and may then be introduced into a patient in positions appropriate for the amelioration of the symptoms of an FBP disorder or a proliferative or differentiative disorders, *e.g.*, cancer and tumorigenesis. Alternately, cells that express an unimpaired FBP gene and that are from a MHC matched individual can be utilized, and may include, for example, brain cells. The expression of the FBP gene sequences is controlled by the appropriate gene regulatory sequences to allow such expression in the necessary cell types. Such gene regulatory sequences are well known to the skilled artisan. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, U.S. Patent No. 5,399,349.

When the cells to be administered are non-autologous cells, they can be administered using well known techniques that prevent a host immune response against the introduced cells from developing. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Additionally, compounds, such as those identified via techniques such as those described, above, in Section 5.5, that are capable of modulating FBP gene product activity can be administered using standard techniques that are well known to those of skill in the art. In instances in which the compounds to be administered are to involve an interaction with brain cells, the administration techniques should include well known ones that allow for a crossing of the blood-brain barrier.

5.7.3 TARGET PROLIFERATIVE CELL DISORDERS

With respect to specific proliferative and oncogenic disease associated with ubiquitin ligase activity, the diseases that can be treated or prevented by the methods of the present invention include but are not limited to: human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct

carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

Diseases and disorders involving a deficiency in cell proliferation or in which cell proliferation is desired for treatment or prevention, and that can be treated or prevented by inhibiting FBP function, include but are not limited to degenerative disorders, growth deficiencies, hypoproliferative disorders, physical trauma, lesions, and wounds; for example, to promote wound healing, or to promote regeneration in degenerated, lesioned or injured tissues, *etc.* In a specific embodiment, nervous system disorders are treated. In another specific embodiment, a disorder that is not of the nervous system is treated.

5.8 PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

The compounds that are determined to affect FBP gene expression or gene product activity can be administered to a patient at therapeutically effective doses to treat or ameliorate a cell proliferative disorder. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of such a disorder.

5.8.1 EFFECTIVE DOSE

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

5.8.2 FORMULATIONS AND USE

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

6. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF NOVEL UBIQUITIN LIGASE F-BOX PROTEINS AND GENES

The following studies were carried out to identify novel F-box proteins which may act to recruit novel specific substrates to the ubiquitination pathways. Studies involving several organisms have shown that some FBPs play a crucial role in the controlled degradation of important cellular regulatory proteins (*e.g.*, cyclins, cdk-inhibitors, β-catenin, IKBa, *etc.*). These FBPs are subunits of ubiquitin protein SCF ligases formed by three basic subunits: a cullin subunit (called Cdc53 in S. cerevisiae and Cul1 in humans); Skp1; and one of many FBPs. SCF ligases target ubiquitin conjugating enzymes (either Ubc3 or Ubc4) to specific substrates which are recruited by different FBPs. Schematically, the Ubc is bound to the ligase through the cullin subunit while the substrate interacts with the FBP subunit. Although FBPs can bind the cullin subunit directly, the presence of fourth subunit, Skp1, which simultaneously can bind the cullin N-terminus and the F-box of the FBP, stabilizes the complex. Thus, the substrate specificity of the ubiquitin ligase complex is provided by the F-box subunit.

6.1 MATERIALS AND METHODS USED FOR THE IDENTIFICATION AND CHARACTERIZATION OF NOVEL F-BOX GENES

Yeast Two-Hybrid Screening In order to clone the human genes encoding F-box proteins, proteins associated with Skp1 were identified using a modified yeast 2-hybrid system (Vidal et al., 1996, Proc. Nat. Acad. Sci., 93:10315-20; Vidal et al., 1996, Proc. Nat. Acad. Sci., 93:10321-26). This modified system takes advantage of using three reporter genes expressed from three different Gal4 binding site promoters, thereby decreasing the number of false positive interactions. This multiple reporter gene assay facilitates identification of true interactors.

Human Skp1 was used as a bait to search for proteins that interact with Skp1, such as novel F-box proteins and the putative human homolog of Cdc4. The plasmids pPC97-CYH2 and pPC86 plasmids, encoding the DNA binding domain (DB, aa 1 - 147) and the transcriptional activation domain (AD, aa 768 - 881) of yeast GAL4, and containing LEU2 and TRP1 as selectable markers, respectively, were used (Chevray and Nathans, 1992, Proc. Nat. Acad. Sci., 89:5789-93; Vidal et al., *supra*).

An in-frame fusion between Skp1 and DB was obtained by homologous recombination of the PCR product described below. The following 2 oligonucleotides were designed and obtained as purified primers from Gene Link Inc.: 5'-AGT-AGT-AAC-AAA-GGT-CAA-AGA-CAG-TTG-ACT-GTA-TCG-TCG-AGG-ATG-CCT-TCA-ATT-AAG-TT (SEQ ID NO: 80); 3'-GCG-GTT-ACT-TAC-TTA-GAG-CTC-GAC-GTC-TTA-

CTT-ACT-TAG-CTC-ACT-TCT-CTT-CAC-ACC-A (SEQ ID NO: 81). The 5' primer corresponds to a sequence located in the DB of the pPC97-CYH2 plasmid (underlined) flanked by the 5' sequence of the skp1 gene. The 3' primer corresponds to a sequence located by polylinker of the pPC97-CYI12 plasmid (underlined) flanked by the 3' sequence of the skp1 gene. These primers were used in a PCR reaction containing the following components: 100 ng DNA template (skp1 pET plasmid), 1 μM of each primer, 0.2 mM dNTP, 2 mM $MgCl_2$, 10 mM KCl, 20 mM TrisCl pH 8.0, 0.1% Triton X-100, 6 mM $(NH_4)_2SO_4$, 10 μg/ml nuclease-free BSA, 1 unit of Pfu DNA polymerase (4' at 94°C, 1' at 50 C, 10' at 72 °C for 28 cycles). Approximately 100 ng of PCR product were transformed into yeast cells (MaV103 strain; Vidal et al., 1996, Proc. Natl. Acad. Sci. U.S.A. 93:10315-10320; Vidal et al., 1996, Proc. Natl. Acad. Sci. U.S.A. 93:10321-10326) in the presence or in the absence of 100 ng of pPC97-CYH2 plasmid previously digested with BglII and SalI. As a result of the homologous recombination, only yeast cells containing the pPC97-CYH2 plasmid homologously recombined with skp1 cDNA, grew in the absence of leucine. Six colonies were isolated and analyzed by immunoblotting for the expression of Skp1, as described (Vidal et al., *supra*). All 6 colonies, but not control colonies, expressed a Mr 36,000 fusion-protein that was recognized by our affinity purified anti-Skp1 antibody.

The AD fusions were generated by cloning cDNA fragments in the frame downstream of the AD domains and constructs were confirmed by sequencing, immunoblot, and interaction with Skp1. The pPC86-Skp2s (pPC86) include: pPC86-Skp2, and pPC86-Skp2-CT (aa 181-435 of Skp2). The first fusion represents our positive control since Skp2 is a known interactor of Skp1 (Zhang, et al, 1995, Cell, 82: 915-25); the latter fusion was used as a negative control since it lacked the F-box required for the interaction with Skp1.

MaV103 strain harboring the DB-skp1 fusions was transformed with an activated T-cell cDNA library (Alala 2; Hu, et al., Genes & Dev. 11: 2701-14) in pPC86 using the standard lithium acetate method. Transformants were first plated onto synthetic complete (SC)-Leu-Trp plates, followed by replica plating onto (SC)-Leu-Trp-His plates containing 20 mM 3-aminotriazole (3-AT) after 2 days. Yeast colonies grown out after additional 3-4 days of incubation were picked as primary positives and further tested in three reporter assays: i) growth on SC-Leu-Trp-His plates supplemented with 20 mM 3-AT; ii) -galactosidase activity; and iii) URA3 activation on SC-Leu-Trp plates containing 0.2% 5-fluoroortic acid, as a counterselection method. Of the 3 x 10^6 yeast transformants screened AD plasmids were rescued from the fifteen selected positive colonies after all three. MaV103 cells were re-transformed with either rescued AD plasmids and the DBskp1 fusion or rescued AD plasmid and the pPC97-CYH2vector without a cDNA insert as control. Eleven AD plasmids from colonies that repeatedly tested positive in all three

reporter assays (very strong interactors) and four additional AD plasmids from clones that were positive on some but not all three reporter assays (strong interactors) were recovered and sequenced with the automated ABI 373 DNA sequencing system.

Cloning of full length FBPs Two of the clones encoding FBP4 and FBP5 appeared to be full-length, while full length clones of 4 other cDNAs encoding FBP1, FBP2, FBP3 and FBP7 were obtained with RACE using Marathon-Ready cDNA libraries (Clonthec, cat. # 7406, 7445, 7402) according to the manufacturer's instructions. A full-length clone encoding FBP6 was not obtained. Criteria for full length clones included at least two of the following: i) the identification of an ORF yielding a sequence related to known F-box proteins; ii) the presence of a consensus Kozak translation initiation sequence at a putative initiator methionine codon; iii) the identification of a stop codon in the same reading frame but upstream of the putative initiation codon; iv) the inability to further increase the size of the clone by RACE using three different cDNA libraries.

Analysis by Immunoblotting of Protein from Yeast Extracts Yeast cells were grown to mid-logarithmic phase, harvested, washed and resuspended in buffer (50 mM Tris pH 8.0, 20% glycerol, 1 mM EDTA, 0.1% Triton X-100, 5 mM MgCl2, 10 mM ß-mercaptoethanol, 1 mM PMSF, 1 mg/ml Leupeptin, 1 mg/ml Pepstatin) at a cell density of about 109 cells/ml. Cells were disrupted by vortexing in the presence of glass beads for 10 min at 40C. Debris was pelleted by centrifugation at 12,000 RPM for 15 min at 40C. Approximately 50 g of proteins were subjected to immunoblot analysis as described (Vidal et al., 1996a, *supra*; Vidal et al., 1996b, *supra*).

DNA database searches and analysis of protein motifs ESTs (expressed sequence tags) with homology to FBP genes were identified using BLAST, PSI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and TGI Sequence Search (http://www.tigr.org/cgi-bin/BlastSearch/blast_tgi.cgi). ESTs that overlapped more than 95 % in at least 100 bps were assembled into novel contiguous ORFs using Sequencher 3.0. Protein domains were identified with ProfileScan Server (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html), BLOCKS Sercher (http://www.blocks.fhcrc.org/blocks_search.html) and IMB Jena (http://genome.imb-jena.de/cgi-bin/GDEWWW/menu.cgi).

Construction of F-box mutants Delta-F-box mutants [(ΔF)FBP1, residues 32-179; (ΔF)FBP2, residues 60-101; (ΔF)FBP3a, residues 40-76; (ΔF)FBP4, residues 55-98] were

obtained by deletion with the appropriate restriction enzymes with conservation of the reading frame. (ΔF)Skp2 mutant was obtained by removing a DNA fragment (nucleotides 338-997) with BspEI and XbaI restriction enzymes, and replacing it with a PCR fragment containing nucleotides 457 to 997. The final construct encoded a protein lacking residues 113-152. The leucine 51-to-alanine FBP3a mutant [FBP3a(L51A)] and the tryptophan 76-to-alanine FBP3a mutant [FBP3a(W76A)] were generated by oligonucleotide-directed mutagenesis using the polymerase chain reaction of the QuikChange site-directed mutagenesis kit (Stratagene). All mutants were sequenced in their entirety.

Recombinant proteins cDNA fragments encoding the following human proteins: Flag-tagged FBP1, Flag-tagged (ΔF)FBP1, Flag-tagged FBP3a, Skp2, HA-tagged Cul1, HA-tagged Cul2, (β-catenin, His-tagged cyclin D1, Skp1, His-tagged Skp1, His-tagged Elongin C were inserted into the baculovirus expression vector pBacpak-8 (Clonetech) and cotransfected into Sf9 cells with linearized baculovirus DNA using the BaculoGold transfection kit (Pharmingen). Recombinant viruses were used to infect 5B cells and assayed for expression of their encoded protein by immunoblotting as described above. His-proteins were purified with Nickel-agarose (Invitrogen) according to the manufacturer's instructions.

Antibodies. Anti-Cul1 antibodies was generated by injecting rabbits and mice with the following amino acid peptide: (C)DGEKDTYSYLA (SEQ ID NO: 82). This peptide corresponds to the carboxy-terminus of human Cul1 and is not conserved in other cullins. Anti-Cul2 antibodies was generated by injecting rabbits with the following amino acid peptide: (C)ESSFSLNMNFSSKRTKFKITTSMQ (SEQ ID NO: 83). This peptide is located 87 amino acids from the carboxy-terminus of human Cul2 and is not conserved in other cullins. The anti-Skp1 antibody was generated by injecting rabbits with the peptide (C)EEAQVRKENQW (SEQ ID NO: 84), corresponding to the carboxy-terminus of human Skp1. The cysteine residues (C) were added in order to couple the peptides to keyhole limpet hemocyanin (KLH). All of the antibodies were generated, affinity-purified (AP) and characterized as described (Pagano, M., ed., 1995, "From Peptide to Purified Antibody", in Cell Cycle: Materials and Methods, Spring-Verlag, 217-281). Briefly, peptides whose sequence showed high antigenic index (high hydrophilicity, good surface probability, good flexibility, and good secondary structure) were chosen. Rabbits and mice were injected with peptide-KLH mixed with complete Freund's adjuvant. Subsequently they were injected with the peptide in incomplete Freund's adjuvant, every 2 weeks, until a significant immunoreactivity was detected by immunoprecipitation of 35S-methionine labeled HeLa

extract. These antisera recognized bands at the predicted size in both human extracts and a extracts containing recombinant proteins.

Monoclonal antibody (Mab) to Ubc3 was generated and characterized in collaboration with Zymed Inc. Mab to cyclin B (cat # sc-245) was from Santa Cruz; Mabs to p21 (cat # C24420) and p27 (cat # K25020) from Transduction lab. (Mabs) cyclin E, (Faha, 1993, J. of Virology 67: 2456); AP rabbit antibodies to human p27, Skp2, Cdk2 (Pagano, 1992, EMBO J. 11: 761), and cyclin A (Pagano, 1992, EMBO J. 11: 761), and phospho-site p27 specific antibody, were obtained or generated by standard methods. Where indicated, an AP goat antibody to an N-terminal Skp2 peptide (Santa Cruz, cat # sc-1567) was used. Rat anti-HA antibody was from Boehringer Mannheim (cat. #1867423), rabbit anti-HA antibody was from Santa Cruz (cat. # sc-805), mouse anti-Flag antibody was from Kodak (cat. # IB13010), rabbit anti-Flag antibody was from Zymed (cat. #71-5400), anti-Skp1 and anti-(β-catenin mouse antibodies were from Transduction Laboratories (cat. # C19220 and P46020, respectively). The preparation, purification and characterization of a Mab to human cyclin D1 (clone AM29, cat. #33-2500) was performed in collaboration with Zymed Inc. Antiserum to human cyclin D1 was produced as described(Ohtsubo et al., 1995, Mol Cell Biol, 15, 2612-2624).

Extract preparation and cell synchronization Protein extraction was performed as previously described (Pagano, 1993, J. Cell Biol. 121: 101) with the only difference that 1 µm okadaic acid was present in the lysis buffer. Human lung fibroblasts IMR-90 were synchronized in G0/G1 by serum starvation for 48 hours and the restimulated to re-enter the cell cycle by serum readdition. HeLa cells were synchronized by mitotic shake-off as described (Pagano, 1992, EMBO J. 11: 761). Synchronization was monitored by flow cytometry. For in vitro ubiquitination and degradation assays, G1 HeLa cells were obtained with a 48-hour lovastatin treatment and protein extraction performed as described below.

Immunoprecipitation and Immunoblotting. Cell extracts were prepared by addition of 3-5 volumes of standard lysis buffers (Pagano et al., 1992, Science 255, 1144-1147), and conditions for immunoprecipitation were as described (Jenkins and Xiong, 1995; Pagano et al., 1992a Science 255-1144-1147). Proteins were transfered from gel to a nitrocellulose membrance (Novex) by wet blotting as described (Tam et al., 1994 Oncogene 9, 2663). Filters were subjected to immunoblotting using a chemiluminescence (DuPont-NEN) detection system according to the manufacturer's instructions

Protein extraction for in vitro ubiquitination assay Logarithmically growing, HeLa-S3 cells were collected at a density of 6x105 cells/ml. Approx. 4 ml of HeLa S3 cell pellet were suspended in 6 ml of ice-cold buffer consisting of 20 mM Tris-HCl (pH 7.2), 2 mM DTT, 0.25 mM EDTA, 10 µg/ml leupeptin, and 10 µg/ml pepstatin. The suspension was transferred to a cell nitrogen-disruption bomb (Parr, Moline, IL, cat #4639) that had been rinsed thoroughly and chilled on ice before use. The bomb chamber was connected to a nitrogen tank and the pressure was brought slowly to 1000 psi. The chamber was left on ice under the same pressure for 30 minutes and then the pressure was released slowly. The material was transferred to an Eppendorf tube and centrifuged in a microcentrifuge at 10,000 g for 10 minutes. The supernatant (S-10) was divided into smaller samples and frozen at -800C.

In vitro ubiquitination The ubiquitination assay was performed as described (Lyapina, 1998, Proc Natl Acad Sci U S A, 95: 7451). Briefly, immuno-beads containing Flag-taggcd FBPs immunoprecipitated with anti-Flag antibody were added with purified recombinant human E1 and E2 enzymes (Ubc2, Ubc3 or Ubc4) to a reaction mix containing biotinylated-ubiquitin. Samples were then analyzed by blotting with HRP-streptavidin. E1 and E2 enzymes and biotinylated-ubiquitin were produced as described (Pagano, 1995, Science 269: 682).

Transient transfections cDNA fragments encoding the following human proteins: FBP1, (ΔF)FBP1, FBP2, (ΔF)FBP2, FBP3a, (ΔF)FBP3a, FBP3a(L51A), FBP3a(W76A), FBP4, (ΔF)FBP4, Skp2, (ΔF)Skp2, HA-tagged β-catenin, untagged β-catenin, Skp1, cyclin D1 were inserted into the mammalian expression vector pcDNA3 (Invitrogen) in frame with a Flag-tag at their C-terminus. Cells were transfected with FuGENE transfection reagent (Boehringer, cat. #1-814-443) according to the manufacture's instruction.

Immunofluorescence Transfected cell monolayers growing on glass coverslips were rinsed in PBS and fixed with 4% paraformaldehyde in PBS for 10 minutes at 4°C followed by permeabilization for 10 minutes with 0.25% Triton X-100 in PBS. Other fixation protocols gave comparable results. Immunofluorescence stainings were performed using 1 µg/ml rabbit anti-Flag antibody as described (Pagano, 1994, Genes & Dev., 8:1627).

Northern Blot Analysis Northern blots were performed using human multiple-tissue mRNAs from Clontech Inc. Probes were radiolabeled with [alpha-32P] dCTP (Amersham Inc.) using a random primer DNA labeling kit (Gibco BRL) (2 x 106 cpm/ml). Washes

were performed with 0.2 x SSC, 0.1% SDS, at 55 - 60°C. FBP1 and FBP3a probes were two HindIII restriction fragments (nucleotides 1 - 571 and 1 - 450, respectively), FBP2, FBP4, and FBP1 probes were their respective full-length cDNAs, and β-ACTIN probe was from Clontech Inc.

Fluorescence in situ hybridization (FISH) Genomic clones were isolated by high-stringency screening (65°C, 0.2 x SSC, 0.1 % SDS wash) of a λFIX II placenta human genomic library (Stratagene) with cDNA probes obtained from the 2-hybrid screening. Phage clones were confirmed by high-stringency Southern hybridization and partial sequence analysis. Purified whole phage DNA was labeled and FISH was performed as described (M. Pagano., ed., 1994, in Cell Cycle: Materials and Methods, 29).

6.2 RESULTS

6.2.1 Characterization of novel F-box Proteins and their activity in vivo

An improved version of the yeast two-hybrid system was used to search for interactors of human Skp1. The MaV103 yeast strain harboring the Gal4 DB-Skp1 fusion protein as bait was transformed with an activated T-cell cDNA library expressing Gal4 AD fusion proteins as prey. After initial selection and re-transformation steps, 3 different reporter assays were used to obtain 13 positive clones that specifically interact with human Skp1. After sequence analysis, the 13 rescued cDNAs were found to be derived from 7 different open reading frames all encoding FBPs. These novel FBPs were named as follows: FBP1, shown in Figure 3 (SEQ ID NO:1); FBP2, shown in Figure 4 (SEQ ID NO:3), FBP3a, shown in Figure 5 (SEQ ID NO:5), FBP4, shown in Figure 7 (SEQ ID NO:7), FBP5, shown in Figure 8 (SEQ ID NO:9), FBP6, shown in Figure 9 (SEQ ID NO:11), FBP7, shown in Figure 10 (SEQ ID NO:13). One of the seven FBPs, FBP1 (SEQ ID NO:1) was also identified by others while our screen was in progress (Margottin et al., 1998, Molecular Cell, 1:565-74).

BLAST programs were used to search for predicted human proteins containing an F-box in databases available through the National Center for Biotechnology Information and The Institute for Genomic Research. The alignment of the F-box motifs from these predicted human FBPs is shown in Figure 1. Nineteen previously uncharacterized human FBPs were identified by aligning available sequences (GenBank Accession Nos. AC002428, AI457595, AI105408, H66467, T47217, H38755, THC274684, AI750732, AA976979, AI571815, T57296, Z44228, Z45230, N42405, AA018063, AI751015, AI400663, T74432, AA402415, AI826000, AI590138, AF174602, Z45775, AF174599, THC288870, AI017603, AF174598, THC260994, AI475671, AA768343,

AF174595, THC240016, N70417, T10511, AF174603, EST04915, AA147429, AI192344, AF174594, AI147207, AI279712, AA593015, AA644633, AA335703, N26196, AF174604, AF053356, AF174606, AA836036, AA853045, AI479142, AA772788, AA039454, AA397652, AA463756, AA007384, AA749085, AI640599, THC253263, AB020647, THC295423, AA434109, AA370939, AA215393, THC271423, AF052097, THC288182, AL049953, CAB37981, AL022395, AL031178, THC197682, and THC205131), with the nucleotide sequences derived from the F-box proteins disclosed above.

The nineteen previously uncharacterized FBP nucleotide sequences thus identified were named as follows: FBP3b, shown in Figure 6 (SEQ ID NO:23); FBP8, shown in Figure 11 (SEQ ID NO:25); FBP9, shown in Figure 12 (SEQ ID NO:27); FBP10, shown in Figure 13 (SEQ ID NO:29); FBP11, shown in Figure 14 (SEQ ID NO:31); FBP12, shown in Figure 15 (SEQ ID NO:33); FBP13, shown in Figure 16 (SEQ ID NO:35); FBP14, shown in Figure 17 (SEQ ID NO:37); FBP15, shown in Figure 18 (SEQ ID NO:39); FBP16, shown in Figure 19 (SEQ ID NO:41); FBP17, shown in Figure 20 (SEQ ID NO:43); FBP18, shown in Figure 21 (SEQ ID NO:45); FBP19, shown in Figure 22 (SEQ ID NO:47); FBP20, shown in Figure 23 (SEQ ID NO:49); FBP21, shown in Figure 24 (SEQ ID NO:51); FBP22, shown in Figure 25 (SEQ ID NO:53); FBP23, shown in Figure 26 (SEQ ID NO:55); FBP24, shown in Figure 27 (SEQ ID NO:57); and FBP25, shown in Figure 28 (SEQ ID NO:59). The alignment of the F-box motifs from these predicted human FBPs is shown in Figure 1A. Of these sequences, the nucleotide sequences of fourteen identified FBPs, FBP3b (SEQ ID NO:23), FBP8 (SEQ ID NO:25), FBP11 (SEQ ID NO:31), FBP12 (SEQ ID NO:33), FBP13 (SEQ ID NO:35), FBP14 (SEQ ID NO:37), FBP15 (SEQ ID NO:39), FBP17 (SEQ ID NO:43), FBP18 (SEQ ID NO:45), FBP20 (SEQ ID NO:49), FBP21 (SEQ ID NO:51), FBP22 (SEQ ID NO:53), FBP23 (SEQ ID NO:55), and FBP25 (SEQ ID NO:59) were not previously assembled and represent novel nucleic acid molecules. The five remaining sequences, FBP9 (SEQ ID NO:27), FBP10 (SEQ ID NO:29), FBP16 (SEQ ID NO:41), FBP19 (SEQ ID NO:47), and FBP24 (SEQ ID NO:57) were previously assembled and disclosed in the database, but were not previously recognized as F-box proteins.

Computer analysis of human FBPs revealed several interesting features (see the schematic representation of FBPs in Figure 2. Three FBPs contain WD-40 domains; seven FBPs contain LRRs, and six FBPs contain other potential protein-protein interaction modules not yet identified in FBPs, such as leucine zippers, ring fingers, helix-loop-helix domains, proline rich motifs and SH2 domains.

As examples of the human FBP family, a more detailed characterization of some FBPs was performed. To confirm the specificity of interaction between the novel FBPs and human Skp1, eight in vitro translated FBPs were tested for binding to His-tagged-Skp1 pre-bound to Nickel-agarose beads. As a control Elongin C was used, the only known human Skp1 homolog. All 7 FBPs were able to bind His-Skp1 beads but not to His-tagged-Elongin C beads (Figure 29). The small amount of FBPs that bound to His-tagged-Elongin C beads very likely represents non-specific binding since it was also present when a non-relevant protein (His-tagged-p27) bound to Nickel-agarose beads was used in pull-down assays (see as an example, Figure 29, lane 12).

F-box deletion mutants, (ΔF)FBP1, (ΔF)FBP2, (ΔF)FBP3a, and mutants containing single point mutations in conserved amino acid residues of the F-box, FBP3a(L51A) and FBP3a(W76A) were constructed. Mutants lacking the F-box and those with point mutations lost their ability to bind Skp1 (Figure 29), confirming that human FBPs require the integrity of their F-box to specifically bind Skp1.

In order to determine whether FBP1, FBP2, FBP3a, FBP4 and FBP7 interact with human Skp1 and Cul1 in vivo (as Skp2 is known to do), flag-tagged-FBP1, -(ΔF)FBP1, -FBP2, -(ΔF)FBP2, -FBP3a, -(ΔF)FBP3a, -FBP4 and -FBP7 were expressed in HeLa cells from which cell extracts were made and subjected to immunoprecipitation with an anti-Flag antibody. As detected in immunoblots with specific antibodies to Cul1, Cul2 (another human cullin), and Skp1, the anti-Flag antibody co-precipitated Cul1 and Skp1, but not Cul2, exclusively in extracts from cells expressing wild-type FBPs (Figure 29 and data not shown). These data indicate that as in yeast, the human Skp1/cullin complex forms a scaffold for many FBPs.

The binding of FBPs to the Skp1/Cul1 complex is consistent with the possibility that FBPs associate with a ubiquitin ligation activity. To test this possibility, Flag-tagged were expressed in HeLa cells, FBPs together with human Skp1 and Cul1. Extracts were subjected to immunoprecipitation with an anti-Flag antibody and assayed for ubiquitin ligase activity in the presence of the human ubiquitin-activating enzyme (E1) and a human Ube. All of the wild type FBPs tested, but not FBP mutants, associated with a ubiquitin ligase activity which produced a high molecular weight smear characteristic of ubiquitinated proteins (Figure 30). The ligase activity was N-ethylmalcinide (NEM) sensitive (Figure 30, lane 2) and required the presence of both Ubc4 and E1. Results similar to those with Ubc4 were obtained using human Ubc3, whereas Ubc2 was unable to sustain the ubiquitin ligase activity of these SCFs (Figure 30, lanes 12, 13).

Using indirect immunofluorescence techniques, the subcellular distribution of FBP1, FBP2, FBP3a, FBP4 and FBP7 was studied in human cells. Flag-tagged-versions

of these proteins were expressed in HeLa, U2OS, and 293T cells and subjected to immunofluorescent staining with an anti-Flag antibody. FBP1, FBP4 and FBP7 were found to be distributed both in the cytoplasm and in the nucleus, while FBP2 was detected mainly in the cytoplasm and FBP3a mainly in the nucleus. Figure 32 shows, as an example, the subcellular localization of FBP1, FBP2, FBP3a, FBP4 observed in HeLa cells. The localization of (ΔF)FBP1, (ΔF)FBP2, (ΔF)FBP3a mutants was identical to those of the respective wild-type proteins (Figure 32) demonstrating that the F-box and the F-box-dependent binding to Skp1 do not determine the subcellular localization of FBPs. Immunofluorescence stainings were in agreement with the results of biochemical subcellular fractionation.

6.2.2 Northern Blot Analysis of Novel Ubiquitin Ligase Gene Transcripts

RNA blot analysis was performed on poly(A)+ mRNA from multiple normal human tissues (heart, brain, placenta, lung, liver, skeletal, muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes, see Figure 33). FBP1 mRNA transcripts (a major band of ~7-kb and two minor bands of ~3.5 - and ~2.5 kb) were expressed in all of the 16 human tissues tested but were more prevalent in brain and testis. Testis was the only tissue expressing the smaller FBP1 mRNA forms in amounts equal to, if not in excess of, the 7 kb form. FBP2 transcripts (~7.7-kb and ~2.4-kb) were expressed in all tissues tested, yet the ratio of the FBP2 transcripts displayed some tissue differences. An approximately 4 kb FBP3a transcript was present in all tissues tested and two minor FBP3a forms of approximately 3 kb and 2 kb became visible, upon longer exposure, especially in the testis. An approximately 4.8 kb FBP4 transcript was expressed in all normal human tissues tested, but was particularly abundant in heart and pancreas. Finally, the pattern of expression of the new FBPs was compared to that of FBP1 whose mRNA species (a major band ~4 kb and a minor band of ~8.5 kb) were found in all tissues but was particularly abundant in placenta.

6.2.3 Chromosomal Localization Of The Human FBP Genes

Unchecked degradation of cellular regulatory proteins (*e.g.*, p53, p27, β-catenin) has been observed in certain tumors, suggesting the hypothesis that deregulated ubiquitin ligases play a role in this altered degradation (reviewed in A. Ciechanover, 1998, Embo J, 17: 7151). A well understood example is that of MDM2, a proto-oncogene encoding a ubiquitin ligase whose overexpression destabilize its substrate, the tumor suppressor p53 (reviewed by Brown and Pagano, 1997, Biochim Biophys Acta,1332: 1, 1998). To map the chromosomal localization of the human FBP genes and to determine if

these positions coincided with loci known to be altered in tumors or in inherited disease, fluorescence in situ hybridization (FISH) was used. The FBP1 gene was mapped and localized to 10q24 (Fig. 34A), FBP2 to 9q34 (Figure 34B), FBP3a to 13q22 (Figure 34C), FBP4 to 5p12 (Figure 34D) and FBP5 to 6q25-26 (Figure 34E). FBP genes (particularly FBP1, FBP3a, and FBP5) are localized to chromosomal loci frequently altered in tumors (for references and details see Online Mendelian Inheritance in Man database, http://www3.ncbi.nlm.nih.gov/omim/). In particular, loss of 10q24 (where FBP1 is located) has been demonstrated in approx. 10 % of human prostate tumors and small cell lung carcinomas (SCLC), suggesting the presence of a tumor suppressor gene at this location. In addition, up to 7% of childhood acute T-cell leukemia is accompanied by a translocation involving 10q24 as a breakpoint, either t(10;14)(q24;q11) or t(7;10)(q35;q24). Although rarely, the 9q34 region (where FBP2 is located) has been shown to be a site of loss of heterozygosity (LOH) in human ovarian and bladder cancers. LOH is also observed in the region. Finally, 6q25-26 (where FBP5 is located) has been shown to be a site of loss of heterozygosity in human ovarian, breast and gastric cancers hepatocarcinomas, Burkitt's lymphomas, and parathyroid adenomas.

7. EXAMPLE: FBP1 REGULATES THE STABILITY OF β-CATENIN

Deregulation of β-catenin proteolysis is associated with malignant transformation. *Xenopus* Slimb and *Drosophila* FBP1 negatively regulate the Wnt/ β-catenin signaling pathway (Jiang and Struhl, 1998, *supra*; Marikawa and Elinson, 1998). Since ubiquitin ligase complexes physically associate with their substrates, the studies in this Example were designed to determine whether FBP1 can interact with β-catenin. The results show that FBP1 forms a novel ubiquitin ligase complex that regulates the in vivo stability of β-catenin. Thus, the identification of FBP1 as a component of the novel ubiquitin ligase complex that ubiquitinates β-catenin, provides new targets that can be used in screens for agonists, antagonists, ligands, and novel substrates using the methods of the present invention. Molecules identified by these assays are potentially useful drugs as therapeutic agents against cancer and proliferative disorders.

7.1 MATERIALS AND METHODS FOR IDENTIFICATION OF FBP1 FUNCTION

Recombinant proteins, Construction of F-box mutants, Antibodies, Transient transfections, Immunoprecipitation, Immunoblotting, Cell culture and Extract preparation Details of the methods are described in Section 6.1, *supra*.

7.2 RESULTS

7.2.1 Human FBP1 Interacts With β-Catenin

Flag-tagged FBP1 and β-catenin viruses were used to co-infect insect cells, and extracts were analyzed by immunoprecipitation followed by immunoblotting. β-catenin was co-immunoprecipitated by an anti-Flag antibody (Figure 35A), indicating that in intact cells β-catenin and FBP1 physically interact. It has been shown that binding of the yeast FBP Cdc4 to its substrate Sic1 is stabilized by the presence of Skp1 (Skowyra et al., 1997, Cell, 91, 209-219). Simultaneous expression of human Skp1 had no effect on the strength of the interaction between FBP1 and β-catenin. To test the specificity of the FBP1/β-catenin interaction, cells were co-infected with human cyclin D1 and FBP1 viruses. The choice of this cyclin was dictated by the fact that human cyclin D1 can form a complex with the Skp2 ubiquitin ligase complex (Skp1-Cul1-Skp2; Yu et al., 1998, Proc. Natl. Acad. Sci. U.S.A, 95:11324-9). Under the same conditions used to demonstrate the formation of the FBP1/β-catenin complex, cyclin D1 could not be co-immunoprecipitated with Flag-tagged FBP1, and anti-cyclin D1 antibodies were unable to co-immunoprecipitate FBP1 (Figure 35B, lanes 1-3). Co-expression of Skp1 (Figure 35B, lanes 4-6) or Cdk4 with FBP1 and cyclin D1 did not stimulate the association of cyclin D1 with FBP1.

Mammalian expression plasmids carrying HA-tagged β-catenin and Flag-tagged FBP1 (wild type or mutant) were then co-transfected in human 293 cells. β-catenin was detected in anti-Flag immunoprecipitates when co-expressed with either wild type or (ΔF)FBP1 mutant (Figure 35C, lanes 4-6), confirming the presence of a complex formed between β-catenin and FBP1 in human cells.

7.2.2 F-box Deleted FBP1 Mutant Stabilizes β-Catenin In Vivo

The association of (ΔF)FBP1 to β-catenin suggested that (ΔF)FBP1 might act as a dominant negative mutant in vivo by being unable to bind Skp1/Cul1 complex, on the one hand, while retaining the ability to bind β-catenin, on the other. HA-tagged β-catenin was co-expressed together with Flag-tagged (ΔF)FBP1 or with another F-box deleted FBP, (ΔF)FBP2. FBP2 was also obtained with our screening for Skp1-interactors; and, like FBP1, contains several WD-40 domains. The presence of (ΔF)FBP1 specifically led to the accumulation of higher quantities of β-catenin (Figure 36A). To determine whether this accumulation was due to an increase in β-catenin stability, we measured the half-life of β-catenin using pulse chase analysis. Human 293 cells were transfected with HA-tagged β-catenin alone or in combination with the wild type or mutant FBP1. While

wild type Fpb1 had little effect on the degradation of β-catenin, the F-box deletion mutant prolonged the half life of β-catenin from 1 to 4 hours (Figure 36B).

FBP1 is also involved in CD4 degradation induced by the HIV-1 Vpu protein (Margottin et al., *supra*). It has been shown that Vpu recruits FBP1 to DC4 and (ΔF) FBP1 inhibits Vpu-mediated CD4 regulation. In addition, FBP1-ubiquitin ligase complex also controls the stability of IKBαs (Yaron et al., 1998, Nature, 396: 590). Thus, the interactions between FBP1 and β-catenin, Vpu protein, CD4, and IKBαs are potential targets that can be used to screen for agonists, antagonists, ligands, and novel substrates using the methods of the present invention.

8. EXAMPLE: METHODS FOR IDENTIFYING p27 AS A SUBSTRATE OF THE FBP Skp2

Degradation of the mammalian G1 cyclin-dependent kinase (Cdk) inhibitor p27 is required for the cellular transition from quiescence to the proliferative state. The ubiquitination and degradation of p27 depend upon its phosphorylation by cyclin/Cdk complexes. Skp2, an F-box protein essential for entry into S phase, specifically recognizes p27 in a phosphorylation-dependent manner. Furthermore, both in vivo and in vitro, Skp2 is a rate-limiting component of the machinery that ubiquitinates and degrades phosphorylated p27. Thus, p27 degradation is subject to dual control by the accumulation of both Skp2 and cyclins following mitogenic stimulation.

This Example discloses novel assays that have been used to identify the interaction of Skp2 and p27 in vitro. First, an in vitro ubiquitination assay performed using p27 as a substrate is described. Second, Skp2 is depleted from cell extracts using anti-Skp2 antibody, and the effect on p27 ubiquitin ligase activity is assayed. Purified Skp2 is added back to such immunodepleted extracts to restore p27 ubiquitination and degradation. Also disclosed is the use of a dominant negative mutant, (ΔF)Skp2, which interferes with p27 ubiquitination and degradation.

The assays described herein can be used to test for compounds that inhibit cell proliferation. The assays can be carried out in the presence or absence of molecules, compounds, peptides, or other agents described in Section 5.5. Agents that either enhance or inhibit the interactions or the ubiquitination activity can be identified by an increase or decrease the formation of a final product are identified. Such agents can be used, for example, to inhibit Skp2-regulated p27 ubiquitination and degradation in vivo. Molecules identified by these assays are potentially useful drugs as therapeutic agents against cancer and proliferative disorders.

Dominant negative mutants, for example the mutant (ΔF)Skp2, and antisense oligos targeting SKP2, mRNA interfere with p27 ubiquitination and degradation, and can be used in gene therapies against cancer. The assays described herein can also be used to identify novel substrates of the novel FBP proteins, as well as modulators of novel ubiquitin ligase complex - substrate interactions and activities.

8.1 MATERIALS AND METHODS FOR IDENTIFICATION OF p27 AS A Skp2 SUBSTRATE

Protein extraction for in vitro ubiquitination assay Approx. 4 ml of HeLa S3 cell pellet were suspended in 6 ml of ice-cold buffer consisting of 20 mM Tris-HCl (pH 7.2), 2 mM DTT, 0.25 mM EDTA, 10 μg/ml leupeptin, and 10 μg/ml pepstatin. The suspension was transferred to a cell nitrogen-disruption bomb (Parr, Moline, IL, cat #4639) that had been rinsed thoroughly and chilled on ice before use. The bomb chamber was connected to a nitrogen tank and the pressure was brought slowly to 1000 psi. The chamber was left on ice under the same pressure for 30 minutes and then the pressure was released slowly. The material was transferred to an Eppendorf tube and centrifuged in a microcentrifuge at 10,000 g for 10 minutes. The supernatant (S-10) was divided into smaller samples and frozen at -80°C . This method of extract preparation based on the use of a cell nitrogen-disruption bomb extract preserves the activity to in vitro ubiquitinate p27 better than the method previously described (Pagano et al., 1995, Science 269:682-685).

Reagents and antibodies Ubiquitin aldehyde (Hershko & Rose, 1987, Proc. Natl. Acad. Sci. USA 84:1829-33), methyl-ubiquitin (Hershko & Heller, 1985, Biochem. Biophys. Res. Commun. 128:1079-86) and p13 beads (Brizuela et al., 1987, EMBO J. 6:3507-3514) were prepared as described. β, γ-imidoadenosine-50-triphosphate (AMP-PNP), staurosporine, hexokinase, and deoxy-glucose were from Sigma; lovastatine obtained from Merck; flavopiridol obtained from Hoechst Marion Roussel. The phospho-site p27 specific antibody was generated in collaboration with Zymed Inc. by injecting rabbits with the phospho-peptide NAGSVEQT*PKKPGLRRRQT (SEQ ID NO: 85), corresponding to the carboxy terminus of the human p27 with a phosphothreonine at position 187 (T*). The antibody was then purified from serum with two rounds of affinity chromatography using both phospho- and nonphospho-peptide chromatography. All the other antibodies are described in Section 6.1.

Immunodepletion Assays For immunodepletion assays, 3 μl of an Skp2 antiserum was adsorbed to 15 μl Affi-Prep Protein-A beads (BioRad), at 4°C for 90 min. The beads were

washed and then mixed (4°C, 2 hours) with 40 µl of HeLa extract (approximately 400 µg of protein). Beads were removed by centrifugation and supernatants were filtered through a 0.45-µ Microspin filter (Millipore). Immunoprecipitations and immunoblots were performed as described (M. Pagano, et al., 1995, *supra*. Rabbit polyclonal antibody against purified GST-Skp2 was generated, affinity-purified (AP) and characterized as described (M. Pagano, in Cell Cycle-Materials and Methods , M. Pagano Ed. (Springer, NY, 1995), chap. 24; E. Harlow and D. Lane, in Using antibodies. A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1998), in collaboration with Zymed Inc. (cat # 51-1900). Monoclonal antibodies (Mabs) to human Cul1, and cyclin E, (Faha et al., 1993, J. of Virology 67:2456); AP rabbit antibodies to human p27, Skp1 (Latres et al., 1999, Oncogene 18:849), Cdk2 (Pagano, et al., 1992, Science 255:1144) and phospho-site p27 specific antibody. Mab to cyclin B was from Santa Cruz (cat # sc-245); Mabs to p21 (cat # C24420) and p27 (cat # K25020) Transduction lab; anti-Flag rabbit antibody from Zymed (cat # 71-5400). An AP goat antibody to an N-terminal Skp2 peptide (Santa Cruz, cat # sc-1567) was used.

Construction of Skp2 F-box mutant (ΔF)Skp2 mutant was obtained by removing a DNA fragment (nucleotides 338-997) with BspEI and XbaI restriction enzymes, and replacing it with a PCR fragment containing nucleotides 457 to 997. The final construct encoded a protein lacking residues 113-152.

Recombinant proteins cDNA fragments encoding the following human proteins: Flag-tagged FBP1, Flag-tagged (ΔF)FBP1, Flag-tagged FBP3a, Skp2, HA-tagged Cul1, HA-tagged Cul2, β-catenin, His-tagged cyclin D1, Skp1, His-tagged Skp1, His-tagged Elongin C were inserted into the baculovirus expression vector pBacpak-8 (Clonetech) and cotransfected into Sf9 cells with linearized baculovirus DNA using the BaculoGold transfection kit (Pharmingen). Baculoviruses expressing human His-tagged cyclin E and HA-tagged Cdk2 were supplied by D. Morgan (Desai, 1992, Molecular Biology of the Cell 3: 571). Recombinant viruses were used to infect 5B cells and assayed for expression of their encoded protein by immunoblotting as described above. His-proteins were purified with Nickel-agarose (Invitrogen) according to the manufacturer's instructions. The different complexes were formed by co-expression of the appropriate baculoviruses and purified by nickel-agarose chromatography, using the His tag at the 5' of Skp1 and cyclin E. Unless otherwise stated, recombinant proteins were added to incubations at the following amounts: cyclin E/Cdk2, ~0.5 pmol; Skp1, ~0.5 pmol; Skp2, ~0.1 pmol; FBP1, ~0.1 pmol; FBP3a,

~0.1 pmol, Cul1, ~0.1 pmol. The molar ratio of Skp1/Skp2, Skp1/FBP1, Skp1/FBP3a, and Skp1/Cul1 in the purified preparations was ~5.

Extract preparation and cell synchronization, Transient transfections, Immunoprecipitation and Immunoblotting Methods were carried out as described in Section 6.1, *supra*.

8.2 RESULTS

8.2.1 p27 IN VITRO UBIQUITINATION ASSAY

In an exemplary in vitro ubiquitination assay, logarithmically growing, HeLa-S3 cells were collected at a density of 6x105 cells/ml. Cells are arrested in G1 by 48-hour treatment with 70 μM lovastatin as described (O'Connor &. Jackman, 1995 in Cell Cycle-Materials and Methods, M. Pagano, ed., Springer, NY, chap. 6). 1 μl of in vitro translated [35S]p27 is incubated at 30°C for different times (0 - 75 minutes) in 10 μl of ubiquitination mix containing: 40 mM Tris pH 7.6, 5 mM $MgCl_2$, 1 mM DTT, 10 % glycerol, 1 μM ubiquitin aldehyde, 1 mg/ml methyl ubiquitin, 10 mM creatine phosphate, 0.1 mg/ml creatine phosphokinase, 0.5 mM ATP, 1 μM okadaic acid, 20-30 μg HeLa cell extract. Ubiquitin aldehyde can be added to the ubiquitination reaction to inhibit the isopeptidases that would remove the chains of ubiquitin from p27. Addition of methyl ubiquitin competes with the ubiquitin present in the cellular extracts and terminates p27 ubiquitin chains. Such chains appear as discrete bands instead of a high molecular smear. These shorter polyubiquitin chains have lower affinity for the proteasome and therefore are more stable. Reactions are terminated with Laemmli sample buffer containing β-mercaptoethanol and the products can be analyzed on protein gels under denaturing conditions.

Polyubiquitinated p27 forms are identified by autoradiography. p27 degradation assay is performed in a similar manner, except that (i) Methylated ubiquitin and ubiquitin aldehyde were omitted; (ii) The concentration of HeLa extract is approximately 7 μg/μl; (iii) Extracts are prepared by hypotonic lysis (Pagano et al., 1995, Science 269:682), which preserves proteasome activity better than the nitrogen bomb disruption procedure. In the absence of methyl ubiquitin, p27 degradation activity, instead of p27 ubiquitination activity, can be measured.

The samples are immunoprecipited with an antibody to p27 followed by a subsequent immunoprecipitation with an anti-ubiquitin antibody and run on an 8% SDS gel. The high molecular species as determined by this assay are ubiquitinated. As a control, a p27 mutant lacking all 13 lysines was used. This mutant form of p27 is not ubiquitinated and runs at higher molecular weight on the 8% SDS gel.

8.2.2 p27-Skp2 INTERACTION ASSAYS AND p27-Skp2 IMMUNODEPLETION ASSAY

The recruitment of specific substrates by yeast and human FBPs to Skp1/cullin complexes is phosphorylation-dependent. Accordingly, peptides derived from IκBα and β-catenin bind to FBP1 specifically and in a phosphorylation-dependent manner (Yaron, 1998, Nature 396: 590; Winston et al., 1999, Genes Dev. 13: 270). A p27 phospho-peptide with a phosphothreonine at position 187 was assayed for its ability to bind to human FBPs, including Skp2 and the FBP1, FBP2, FBP3a, FBP4, FBP5, FBP6, and FBP7, isolated by using a 2-hybrid screen using Skp1 as bait, as described in Section 6, above. Four of these FBPs contain potential substrate interaction domains, such as WD-40 domains in FBP1 and FBP2, and leucine-rich repeats in Skp2 and FBP3a. The phospho-p27 peptide was immobilized to Sepharose beads and incubated with these seven in vitro translated FBPs (Figure 37A). Only one FBP, Skp2, was able to bind to the phospho-T187 p27 peptide. Then, beads linked to p27 peptides (in either phosphorylated or unphosphorylated forms) or with an unrelated phospho-peptide were incubated with HeLa cell extracts. Proteins stably associated with the beads were examined by immunoblotting. Skp2 and its associated proteins, Skp1 and Cul1, were readily detected as proteins bound to the phospho-p27 peptide but not to control peptides (Figure 37B).

To further study p27 association to Skp2, in vitro translated p27 was incubated with either Skp1/Skp2 complex, cyclin E/Cdk2 complex, or the combination of both complexes under conditions in which p27 is phosphorylated on T187 by cyclin E/Cdk2 (Montagnoli, A., et al., 1999, Genes & Dev 13: 1181). Samples were then immunoprecipitated with an anti-Skp2 antibody. p27 was co-immunoprecipitated with Skp2 only in the presence of cyclin E/Cdk2 complex (Fig. 37C). Notably, under the same conditions, a T187-to-alanine p27 mutant, p27(T187A), was not co-immunoprecipitated by the anti-Skp2 antibody. Finally, we tested Skp2 and p27 association in vivo. Extracts from HeLa cells and IMR90 human diploid fibroblasts were subjected to immunoprecipitation with two different antibodies to Skp2 and then immunoblotted. p27 and Cul1, but not cyclin D1 and cyclin B1, were specifically detected in Skp2 immunoprecipitates (Fig. 38). Importantly, using a phospho-T187 site p27 specific antibody we demonstrated that the Skp2-bound p27 was phosphorylated on T187 (Fig. 38, lane 2, bottom panel). Furthermore, an anti-peptide p27 antibody specifically co-immunoprecipitated Skp2. These results indicate that the stable interaction of p27 with Skp2 was highly specific and dependent upon phosphorylation of p27 on T187.

A cell-free assay for p27 ubiquitination which faithfully reproduced the cell cycle stage-specific ubiquitination and degradation of p27 has been developed (Montagnoli

et al., supra). Using this assay, a p27-ubiquitin ligation activity is higher in extracts from asynchronously growing cells than in those from G1-arrested cells (Figure 39A, lanes 2 and 4). In accordance with previous findings (Montagnoli, A., et al., supra), the addition of cyclin E/Cdk2 stimulated the ubiquitination of p27 in both types of extracts (Figure 39A, lanes 3 and 5). However, this stimulation was much lower in extracts from G1-arrested cells than in those from growing cells, suggesting that in addition to cyclin E/Cdk2, some other component of the p27-ubiquitin ligation system is rate-limiting in G1. This component could be Skp2 since, in contrast to other SCF subunits, its levels are lower in extracts from G1 cells than in those from asynchronous cells and are inversely correlated with levels of p27 (Figures 39B and 43). Skp2 was thus tested to determine if it is a rate-limiting component of a p27 ubiquitin ligase activity . The addition of recombinant purified Skp1/Skp2 complex alone to G1 extracts did not stimulate p27 ubiquitination significantly (Figure 39A, lane 6). In contrast, the combined addition of Skp1/Skp2 and cyclin E/Cdk2 complexes strongly stimulated p27 ubiquitination in G1 extracts (Figure 39A, lane 7). Similarly, the combined addition of Skp1/Skp2 and cyclin E/Cdk2 strongly stimulated p27 proteolysis as measured by a degradation assay (Figure 39A, lanes 13-16). Since the Skp1/Skp2 complex used for these experiments was isolated from insect cells co-expressing baculovirus His-tagged-Skp1 and Skp2 (and co-purified by nickel-agarose chromatography), it was possible that an insect-derived F-box protein co-purified with His-Skp1 and was responsible for the stimulation of p27 ubiquitination in G1 extracts. This possibility was eliminated by showing that the addition of a similar amount of His-tagged-Skp1, expressed in the absence of Skp2 in insect cells and purified by the same procedure, did not stimulate p27 ubiquitination in the presence of cyclin E/Cdk2 (Figure 39A, lane 8). Furthermore, we found that neither FBP1 nor FBP3a could replace Skp2 for the stimulation of p27-ubiquitin ligation in G1 extracts (Figure 39A, lanes 9-12). Stimulation of p27-ubiquitination in G1 extracts by the combined addition of Skp1/Skp2 and cyclin E/Cdk2 could be observed only with wild-type p27, but not with the p27(T187A) mutant (lanes 17-20), indicating that phosphorylation of p27 on T187 is required for the Skp2-mediated ubiquitination of p27. These findings indicated that both cyclin E/Cdk2 and Skp1/Skp2 complexes are rate-limiting for p27 ubiquitination and degradation in the G1 phase.

To further investigate the requirement of Skp2 for p27 ubiquitin ligation, Skp2 was specifically removed from extracts of asynchronously growing cells by immunodepletion with an antibody to Skp2. The immunodepletion procedure efficiently removed most of Skp2 from these extracts and caused a drastic reduction of p27-ubiquitin ligation activity (Figure 40A, lane 4) as well as of p27 degradation activity. This effect was specific as shown by the following observations: (i) Similar treatment with pre-immune

serum did not inhibit p27-ubiquitination (Figure 40A, lane 3); (ii) Pre-incubation of anti-Skp2 antibody with recombinant GST-Skp2 (lane 5), but not with a control protein (lane 4), prevented the immunodepletion of p27-ubiquitination activity from extracts; (iii) p27-ubiquitinating activity could be restored in Skp2-depleted extracts by the addition of His-Skp1/Skp2 complex (Figure 40B, lane 3) but not His-Skp1 (lane 2), His-Skp1/Cul1 complex (lane 4), or His-Skp1/FBP1.

We then immunoprecipitated Skp2 from HeLa extracts and tested whether this immunoprecipitate contained a p27 ubiquitinating activity. The anti-Skp2 beads, but not a immunoprecipitate made with a pre-immune (PI) serum, was able to induce p27 ubiquitination in the presence of cyclin E/Cdk2 (Figure 40C, lanes 2 and 3). The addition of purified recombinant E1 ubiquitin-activating enzyme, and purified recombinant Ubc3 did not greatly increase the ability of the Skp2 immunoprecipitate to sustain p27 ubiquitination, (Figure 40C, lane 5), likely due to the presence of both proteins in the rabbit reticulocyte lysate used for p27 in vitro translation.

8.2.3 F-BOX DELETED SKP2 MUTANT STABILIZES p27 IN VIVO

Skp2 also targets p27 for ubiquitin-mediated degradation in vivo. The F-box-deleted FBP1 mutant, (ΔF)FBP1, acts in vivo as a dominant negative mutant, most likely because without the F-box is unable to bind Skp1/Cul1 complex but retains the ability to bind its substrates. Therefore, once expressed in cells, (ΔF)Fb sequesters β-catenin and IKBα and causes their stabilization. An F-box deleted Skp2 mutant, (ΔF)Skp2, was constructed. p27 was expressed in murine cells either alone or in combination with (ΔF)Skp2 or (ΔF)FBP1 (see Figure 41). The presence of (ΔF)Skp2 led to the accumulation of higher quantities of p27. To determine whether this accumulation was due to an increase in p27 stability, the half-life of p27 was measured using pulse chase analysis (for details, see Section 8, above). Indeed, (ΔF)Skp2 prolonged p27 half-life from less than 1 hour to ~3 hours. Since in these experiments the efficiency of transfection was approximately 10%, (ΔF)Skp2 affected only the stability of co-expressed human exogenous p27, but not of murine endogenous p27.

8.2.4 SKP2 ANTISENSE EXPERIMENTS

SKP2 mRNA was targeted with antisense oligonucleotides to determine whether a decrease in Skp2 levels would influence the abundance of endogenous p27. Two different antisense oligos, but not control oligodeoxynucleotides induced a decrease in Skp2 protein levels (Figure 42). Concomitant with the Skp2 decrease, there was a substantial increase in the level of endogenous p27 protein. Similar results were obtained with cells

blocked at the G1/S transition with hydroxyurea or aphidicolin treatment (lanes 9-16). Thus, the effect of the SKP2 antisense oligos on p27 was not a secondary consequence of a possible block in G1 due to the decrease in Skp2 levels.

Antisense experiments were performed as described in (Yu, 1998, Proc. Natl. Acad. Sci. U. S. A. 95: 11324). Briefly, four oligodeoxynucleotides that contain a phosphorothioate backbone and C-5 propyne pyrimidines were synthesized (Keck Biotechnology Resource Laboratory at Yale University): (1) 5'-CCTGGGGGATGTTCTCA-3' (SEQ ID NO: 86) (the antisense direction of human Skp2 cDNA nucleotides 180-196); (2) 5'-GGCTTCCGGGCATTTAG-3' (SEQ ID NO: 87) [the scrambled control of (1)]; (3) 5'-CATCTGGCACGATTCCA-3' (SEQ ID NO: 88) (the antisense direction of Skp2 cDNA nucleotides 1137-1153); (4) 5'-CCGCTCATCGTATGACA-3' (89) [the scrambled control for (3)]. The oligonucleotides were delivered into HeLa cells using Cytofectin GS (Glen Research) according to the manufacturers instructions. The cells were then harvested between 16 and 18 hours postransfection.

9. EXAMPLE: ASSAY TO IDENTIFY AN FBP INTERACTION WITH A CELL CYCLE REGULATORY PROTEIN (*e.g.*, SKP2 with E2F)

The following study was conducted to identify novel substrates of the known FBP, Skp2.

As shown in Figure 44, E2F-1, but not other substrates of the ubiquitin pathway assayed, including p53 and Cyclin B, physically associates with Skp2. Extracts of insect cells infected with baculoviruses co-expressing Skp2 and E2F-1, (lanes 1,4 and 5), or Skp2 and hexa-histidine p53 (His-p53) (lanes 2,6,7,10 and 11), or Skp2 and His-Cyclin B (lanes 3,8,9,12, and 13) were either directly immunoblotted with an anti-serum to Skp2 (lanes 1 - 3) or first subjected to immunoblotted with an anti-serum to Skp2 (lanes 1 - 3) or first subjected to immunoprecipitation with the indicated antibodies and then immunoblotted with an anti-serum to Skp2 (lanes 4 - 13). Antibodies used in the immunoprecipitations are: normal purified mouse immunoglobulins (IgG) (lanc 4,6,10 and 12), purified mouse monoclonal anti-E2F-1 antibody (KH-95, from Santa Cruz) (lane 5), purified mouse monoclonal anti-p53 antibody (DO-1, from Oncogene Science) (lane 7), purified rabbit IgG (lane 8), purified rabbit polyclonal anti-Cyclin B antibody (lane 9), purified mouse monoclonal anti-His antibody (clone 34660, from Qiagen) (lanes 11 and 13).

As shown in Figure 44B, Skp2 physically associates with E2F-1 but not with other substrates of the ubiquitin pathway (p53 and Cyclin B). Extracts of insect cells

infected with baculoviruses co-expressing Skp2 and E2F-1 (lanes 1 - 3), or Skp2 and His-p53 (lanes 4 - 6), or Skp2 and His-Cyclin B (lanes 7 - 9) were either directly immunoblotted with antibodies to the indicated proteins (lanes 1,4 and 7) or first subjected to immunoprecipitation with the indicated anti-sera and then immunoblotted with antibodies to the indicated proteins (lanes 2,3,5,6,8 and 9). Anti-sera used in the immunoprecipitations are: anti-Skp2 serum (lanes 2,5 and 8), and normal rabbit serum (NRS) (lane 3,6 and 9).

As shown in Figure 44C, E2F-1 physically associates with Skp2 but not with another F-box protein (FBP1). Extracts of insect cells infected with baculoviruses co-expressing Skp2 and E2F-1 (lanes 1,3 and 4), or Flag-tagged-FBP1 and E2F-1 (lanes 2,5 and 6) were either directly immunoblotted with a mouse monoclonal anti-E2F-1 antibody (lanes 1 and 2) or first subjected to immunoprecipitation with the indicated antibodies and then immunoblotted with a mouse monoclonal anti-E2F-1 antibody (lanes 3 - 6). Antibodies used in the immunoprecipitations are: anti-Skp2 serum (lanes 3), NRS (lane 4), purified rabbit polyclonal anti-Flag (lane 5), purified rabbit IgG (lane 6).

The methodology used in this example can also be applied to identify novel substrates of any FBP, including, but not limited to, the FBPs of the invention, such as FBP1, FBP2, FBP3a, FBP3b, FBP4, FBP5, FBP6, FBP7, FBP8, FBP9, FBP10, FBP11, FBP12, FBP13, FBP14, FBP15, FBP16, FBP17, FBP18, FBP19, FBP20, FBP21, FBP22, FBP23, FBP24, and FBP25.

The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference for all purposes.

Claims

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence which encodes a protein comprising the amino acid sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60.

2. An isolated nucleic acid molecule which encodes an F-box protein, or a fragment thereof, having a nucleotide sequence that:

a) hybridizes under highly stringent conditions to the nucleotide sequence of SEQ ID NO: 3, 5, 7, 9, 11 or 13; and

b) does not encompass the nucleotide sequences which encode the following known F-box proteins: Cdc4, Grr1, Met30, Skp2, Cyclin F, Elongin A or mouse Md6.

3. An isolated nucleic acid sequence derived from a mammalian genome that:

a) hybridizes under highly stringent conditions to the nucleotide sequence of SEQ ID NO: 3, 5, 7, 9, 11 or 13; and

b) encodes a gene product which contains an F-box motif and binds to Skp1.

4. An isolated nucleic acid molecule which encodes an F-box protein, said nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, or 59.

5. A nucleotide vector containing the nucleotide sequence of Claim 1, 2, 3, or 4.

6. An expression vector containing the nucleotide sequence of Claim 1, 2, 3, or 4 in operative association with a nucleotide regulatory sequence that controls expression of the nucleotide sequence in a host cell.

7. A genetically engineered host cell that contains the nucleotide sequence of Claim 1, 2, 3, or 4 in operative association with a nucleotide regulatory sequence that controls expression of the nucleotide sequence in the host cell.

8. A transgenic animal having cells which harbor a transgene comprising the nucleic acid of Claim 1, 2, 3, or 4.

9. An animal inactivated in the loci comprising the nucleotide sequence of Claim 1, 2, 3, or 4.

10. An isolated F-box protein having the amino acid sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60.

11. An antibody that immunospecifically binds the polypeptide of Claim 10.

12. A method of diagnosing proliferative and differentiative related disorders comprising measuring FBP gene expression in a patient sample.

13. A method for screening compounds useful for the treatment of proliferative and differentiative disorders comprising contacting a compound with a cell expressing an F-box protein having the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60, or a fragment thereof, and its substrate, and detecting a change in the F-box protein activity.

14. The method of Claim 13 wherein the change in the F-box protein activity is detected by detecting a change in the interaction of the F-box protein with one or more proteins.

15. The method of Claim 14 in which one of the one or more proteins is the substrate of the F-box protein.

16. The method of Claim 13 in which at least one of the one or more proteins is a component of the ubiquitin pathway.

17. The method of Claim 13 in which one of the one or more proteins is Skp1.

18. The method of Claim 13 in which the F-box protein is Fbp1 and the substrate is β-catenin or IKBα.

19. The method of Claim 13 wherein the change in the F-box protein activity is detected by detecting a change in the ubiquitination or degradation of the substrate.

20. A method for screening compounds useful for the treatment of proliferative and differentiative disorders comprising contacting a compound with a cell or a cell extract expressing Skp2 and one or more of p27 and E2F, and detecting a change in the activity of Skp2.

21. The method of Claim 20 wherein the change in the activity of Skp2 is detected by detecting a change in the interaction of Skp2 with p27 and E2F.

22. The method of Claim 20 wherein the change in the activity of Skp2 is detected by detecting a change in the ubiquitination or degradation of p27 or E2F.

23. A method for treating a proliferative or differentiative disorder in a mammal comprising administering to the mammal a compound to the mammal that modulates the synthesis, expression or activity of an FBP gene or gene product so that symptoms of the disorder are ameliorated.

24. The method of Claim 23 in which the disorder is breast cancer.

25. The method of Claim 23 in which the disorder is ovarian cancer.

26. The method of Claim 23 in which the disorder is prostate cancer.

27. The method of Claim 23 in which the disorder is small cell lung carcinoma.

FIG.1

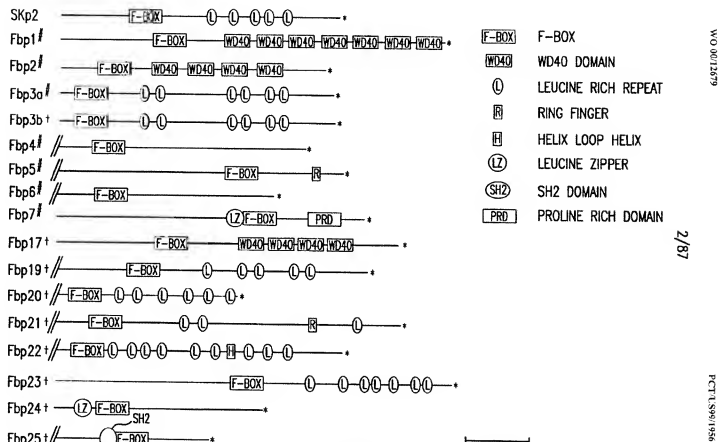
SKp2
Fbp1
Fbp2
Fbp3a
Fbp3b
Fbp4
Fbp5
Fbp6
Fbp7
Fbp17
Fbp19
Fbp20
Fbp21
Fbp22
Fbp23
Fbp24
Fbp25
F-BOX
WD40
SH2
PRO
100AA
F-BOX
WD40 DOMAIN
LEUCINE RICH REPEAT
RING FINGER
HELIX LOOP HELIX
LEUCINE ZIPPER
SH2 DOMAIN
PROLINE RICH DOMAIN

FIG.2

```
        10         20         30         40         50         60
MDPAEAVLQEKALKFMNSSEREDCNNGEPPRKIIPEKNSLRQTYNSCARLCLNQETVCLA

        70         80         90        100        110        120
STAMKTENCVAKTKLANGTSSMIVPKQRKLSASYEKEKELCVKYFEQWSESDQVEFVEHL

       130        140        150        160        170        180
ISQMCHYQHGHINSYLKPMLQRDFITALPARGLDHIAENILSYLDAKSLCAAELVCKEWY

       190        200        210        220        230        240
RVTSDGMLWKKLIERMVRTDSLWRGLAERRGWGQYLFKNKPPDGNAPPNSFYRALYPKII

       250        260        270        280        290        300
QDIETIESNWRCGRHSLQRIHCRSETSKGVYCLQYDDQKIVSGLRDNTIKIWDKNTLECK

       310        320        330        340        350        360
RILTGHTGSVLCLQYDERVIITGSSDSTVRVWDVNTGEMLNTLIHHCEAVLHLRFNNGMM

       370        380        390        400        410        420
VTCSKDRSIAVWDMASPTDITLRRVLVGHRAAVNVVDFDDKYIVSASGDRTIKVWNTSTC

       430        440        450        460        470        480
EFVRTLNGHKRGIACLQYRDRLVVSGSSDNTIRLWDIECGACLRVLEGHEELVRCIRFDN

       490        500        510        520        530        540
KRIVSGAYDGKIKVWDLVAALDPRAPAGTLCLRTLVEHSGRVFRLQFDEFQIVSSSHDDT

       550        560
ILIWDFLNDPAAQAEPPRSPSRTYTYISR
```

FIG.3A

```
                10                  20                  30                  40                  50                  60                  70                  80                  90
TGCGTTGCCTGCGGCCTGCCACCAAGGGCGCCCCCGGAGACGGACCAGTGGCCTGCGCGATTATGCACCCGCCGAGGCGGTGCTGC

           100                 110                 120                 130                 140                 150                 160                 170                 180
AAGAGAAGGCACTCAAGTTTATGCAATTCCTCAGACAGACAAGACTGTAATAATGGCGAACCCCCTAGGAAGATAATACCAGAGAAGAATTCACT

           190                 200                 210                 220                 230                 240                 250                 260                 270                 280
TAGACAGACATACAACAGCTGTGCCAGACTCTGCTTAAACCAAGAAACAGTATGTTTACGAAGCACTGCTATGAAGACTGAGAATTGTGTGCCC

           290                 300                 310                 320                 330                 340                 350                 360                 370
AAAACAAAAACTTGCCAATGGCACTTCCAGTATGATTGTGCCCAAGCAACGGAAACTCTCAGCAAGCTATCAAAAGGAAAAGGAACTGTGTGTCA

           380                 390                 400                 410                 420                 430                 440                 450                 460                 470
AATACTTTGAGCAGTGGTCAGAGTCAAGTGGAATTTGTGGAACATCTTATATCCCAAATGTGTCATTACCAACATGGGCACATAAACTC

           480                 490                 500                 510                 520                 530                 540                 550                 560
GTATCTTAAAGCCTATGTTGCAGACAGCATTTCATAACTGCTCTGCCAGCTGGGGATTGGATCATATGGCTGAGACATTCTGTCATACCTGGAT

           570                 580                 590                 600                 610                 620                 630                 640                 650
GCCAAATCACTATGTGCTGCTGAACTTGTGTGCAAGGAATGGTACCGAGTGCACTGCTGTGGAAGAAGCTTATCGAGAGAATGG

           660                 670                 680                 690                 700                 710                 720                 730                 740                 750
TCAGGACAGATTCTCTGTGGAGAGGCCTGGCAGACCAAGAGGATGGGGACAGTATTTATTCAAAAACAAACCTCCTGCACCGGAATGCTCCTCC

           760                 770                 780                 790                 800                 810                 820                 830                 840
CAACTCTTTTTATACAGCACTTTATCCTAAAATTATACAAGACATTGAGAATCTAATTGGAGATGTGGAAGACATAGTTTACAGAGA

           850                 860                 870                 880                 890                 900                 910                 920                 930                 940
ATTCACTGCCGAGTGAAACAAGGAGTTACTGTTTACAGTATGATGATGACAAAATAGTAAGCGGCTTCGCAGCAACAACAATCAAGA
```

FIG.3B

950 960 970 980 990 1000 1010 1020 1030
TCTGCGATAAAAACACATTCGAATGCAAGCGAATTCTCACAGGCCATACAGGTTCAGTCCTCTGTCTCCAGTATGATGAGAGAGTGATCATAAC

1040 1050 1060 1070 1080 1090 1100 1110 1120
AGGATCATCGGATTCCACGGTCAGAGTGTCGGATGTAAATACAGGTGAAATGCTAAACAACGTTGATTCACCATTGTGAAGCAGTTCTGCACTTG

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
CGTTTCAATAATGGCATGATGGTGACCTGCTCCAAAGATCGTTCCATTGCTGTATGGGATATGCGCCTCCCCAACTGACATTACCCTCCGGAGGG

1230 1240 1250 1260 1270 1280 1290 1300 1310
TGCTGGTCGGACACCGAGCTGCTGTCAATGTTGTAGACTTTGATGACAAGTACATTGTTTCTCCATCTGGGGATAGAACTATAAAGCTATGGAA

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
CACAAGTACTTGTCAATTTGTAAGGACCTTAAATCGACACAAACGAGCCATTGCCTGTTTGCAGTACAGCGGACAGGCTGGTAGTGAGTGGCTCA

1420 1430 1440 1450 1460 1470 1480 1490 1500
TCTGACAACACTATCAGATTATGGGACATAGAATGTGGTGCATGTTTACGAGTGTTAGAAGGCCATGAGGAATTGGTGCGTTGTATTCGATTTG

1510 1520 1530 1540 1550 1560 1570 1580 1590
ATAACAAGACGATAGTCAGTGCGCCCTATGATGCAAAAATTAAAGTGTGGGATCTTGTGCCTGCTTTGGACCCCCGTGCTCCTGCAGGGACACT

1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
CTGTCTACGGACCCTTGTCGAGCATTCCGGAAGAGTTTTTCGACTACAGTTTGATGAATTCCAGATTGTCAGTAGTTCACATGATGACACAATC

FIG.3C

```
1700      1710      1720      1730      1740      1750      1760      1770      1780
CTCATCTGGGACTTCCTAAATGATCCAGCTGCCCAAGCTGAACCCCCCCGTTCCCCTTCTCGGAACATACACCTACATCTCCAGATAAATAACCA
1790      1800      1810      1820      1830      1840      1850      1860      1870      1880
TACACTGACCTCATACTTGCCCAGGACCCATTAAAGTTGCCGTATTTAACGTATCTGCCCAATACCAGGATGAGCAACAACAGTAACAATCAAAC
1890      1900      1910      1920      1930      1940      1950      1960      1970
TACTGCCCAGTTTCCCTGGACTAACCCAGGAGCAGGCCTTTGAGACTCCTGTTGCGACACAGTTGGTCTGCGCCCAGGAACGGTCTACTC
1980      1990      2000      2010      2020      2030      2040      2050      2060
AGCAACAACTGACTGCTTCAGTGCTGCTATCAGAAAGATGTCTTCTATCAATTGTGAATGATTGGAACTTTTAAACCTCCCCTCTCCTCGCTTT
2070      2080      2090      2100      2110      2120      2130      2140      2150
CACCTGTGCACCTAGTTTTTTCCCATTGGTTCCAGACAAAGGTGACTTATAAATATATTTAGTGTTTTGCCAGAAAAAAAAAA
```

FIG.3D

```
        10         20         30         40         50         60
MERKDFETWLDNISVTFLSLTDLQKNETLDHLISLSGAVQLRHLSNNLETLLKRDFLKLL

        70         80         90        100        110        120
PLELSFYLLKWLDPQTLLTCCLVSKQWNKVISACTEVWQTACKNLGWQIDDSVQDALHWK

       130        140        150        160        170        180
KVYLKAILRMKQLEDHEAFETSSLIGHSARVYALYYKDGLLCTGSDDLSAKLWDVSTGQC

       190        200        210        220        230        240
VYGIQTHTCAAVKFDEQKLVTGSFDNTVACWEWSSGARTQHFRGHTGAVFSVDYNDELDI

       250        260        270        280        290        300
LVSGSADFTVKVWALSAGTCLNTLTGHTEWVTKVVLQKCKVKSLLHSPGDYILLSADKYE

       310        320        330        340        350        360
IKIWPIGREINCKCLKTLSVSEDRSICLQPRLHFDGKYIVCSSALGLYQWDFASYDILRV

       370        380        390        400        410        420
IKTPEIANLALGFGDIFALLFDNRYLYIMDLRTESLISRWPLPEYRESKRGSSFLAGEH
```

PG

FIG.4A

```
                10                  20                  30                  40                  50                  60                  70                  80                  90
ATGGACAGAAAGGACTTTGAGACATGGCTGATAACATTTCTTTCTTGACGGACTTGCAGAAAATGAAACTCTGGATCACC

               100                 110                 120                 130                 140                 150                 160                 170                 180
TGATTAGTCTGAGTGGGGCAGTCCAGCTCAGGCATCTCTCCAATAACCTAGAGACTCTCCTCAAGCGGGACTTCCTCAAACTCCTTCCCCTGCA

               200                 210                 220                 230                 240                 250                 260                 270                 280
GCTCAGTTTTATTTGTTAAAATGGCTCGATCCTCAGACTTTACTCACATGCTGCCTCGTCTCTAAACAGTGCAATAAGGTCATAACTGCCTGT

               300                 310                 320                 330                 340                 350                 360                 370
ACAGAGGTGTGGCAGACTGCATGTAAAAATTTGGCCTGGCACATACATGATTCTGTTCAGGACCCTTTGCACTGCAACAAGCTTTATTTGAACG

               390                 400                 410                 420                 430                 440                 450                 460                 470
CTATTTTGCAGAATGAAGCAACTGGAGGACCATCAAGCCTTTCAAACCTCGTCATTAATTGGACACAGTGCCACAGAGTGTATGCACTTTACTACAA

               480                 490                 500                 510                 520                 530                 540                 550                 560
AGATGCACTTCTCTGTACAGGGTCACATGCTGTCTCCAAAGCTGTGGGATGTCAGCACAGGGCAGTGCGTTTATGCCATCCAGACCCACACT

               570                 580                 590                 600                 610                 620                 630                 640                 650
TGTGCACCGCTGAAGTTTGATGAACAGAAGCTTGTGACAAGCTCCTTTGACACACTGTGGCTTGCTGGGAATGCGAGTTCCGGAGCCAGGACCC

               660                 670                 680                 690                 700                 710                 720                 730                 740                 750
AGCAACTTTCGGCGGCACAGGGGGCGGTATTTAGCGTGCACTACAATGATCTTGGTGAGCGGCTCTGCAGCACTTCACTGTGAA

               760                 770                 780                 790                 800                 810                 820                 830                 840
AGTATGGGCTTTATCTGCTGGGACATGCCTGAACACACTCACCGGGCACACGGGAATCGGTCACCAAGGTAGTTTTGCAGAAGTGCAAAGTCAAG

               850                 860                 870                 880                 890                 900                 910                 920                 930                 940
TCTCCTTGCACAGTCCTGCAGACTACATCCTCTTAAGTGCAGACAAATATGAGATTAAGATTTGGCCAATTGCCAGCAGAAATCAACTGTAAGT
```

FIG.4B

```
      950       960       970       980       990      1000      1010      1020      1030
GCTTAAAGACATTGTCTGTCTCTGAGGATAGAAGTATCTGCCTCGAGCCAAGACTTCATTTTGATGTGGCAAATACATTGTCTGTAGTTCAGCACT

     1040      1050      1060      1070      1080      1090      1100      1110      1120
TGGTCTCTACCAGTGGGACTTTGCCAGTTATGATATTCTCAGGGTCATCAAGACTCCTGAGATAGCAAACTTGGCCTTGGCTTTGGAGAT

     1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
ATCTTTGCCCTGCTGTTTGACAACCCCTACCTGTACATCATGGACTTGCCGCAGAGAGCCTGATTAGTCGCTGGCCCTCTGCCAGACTACAGCG

     1230      1240      1250      1260      1270      1280      1290      1300      1310
AATCAAAGAGAGGCTCAAGCTTCCTGGCAGGCCAACATCCTGGCTGAATGGACTGCATGGCAGCAATGACACCGGCTTGGTCTTTGCCCACCAGC

     1320      1330      1340      1350      1360      1370      1380      1390      1400      1410
ATGGCTGACCACAGTATTCACCTGGTGTTGTGCAAGGAGCACCGCCTGACCACCATGACCCACCACGACCCCTGACTTTGGGTGCCGGGCTGCC

     1420      1430      1440      1450      1460      1470
GCTTTTCGGTGCCACCTCTGCCGCACCCACTGCATGAACCAAAGTTCTCACCTAATGGTATCATCA
```

FIG.4C

```
        10         20         30         40         50         60
MKRGGRQSQRNSSEEGTAEKSKKLRTTNEHSQTCDWGNLLQQIILQVFKYLPLLQRAHAS

        70         80         90        100        110        120
QVCRWNQVFHMPQLWRCFEFELNQPATSYLKATHPELIKQIIKRHSNHLQYVSFKVDSS

       130        140        150        160        170        180
KESAEAACQILSQLVNCSLKTLGLISTARPSFMQLPKSHFISALTVVFVNSKSLSSLKIQ

       190        200        210        220        230        240
DTPVDDPSLKVLVANNSDTLKLLKMSSCPHVSPAGILCVADQCHGLRELALNYHLLSDEL

       250        260        270        280        290        300
LLALSSEKHVRLEHLRIDWVSENPGQTHFHTIQKSSWDAFIRHSPKVNLVMYFFLYEEEF

       310        320        330        340        350        360
QPFFRYEIPATHLYFGRSVSKDVLGRVGMTCPRLVELVVCANGLRPLDEELIRIAERCKN

       370        380        390        400        410        420
LSAIGLGECEVSCSAFVEFVKNCGGRLSQLSIMEEVLIPDQKYSLEQIHWEVSKHLGRVW

FPDMAPTW
```

FIG.5A

```
        10         20         30         40         50         60         70         80         90
CGGGGTGCTGTGTGGGGGAAGCCGCCCCCGGCAGCAGGATCAAACGAGGAGCAAGACATAGTCACCGTAATTCATCAGAAGAAGCAACTGCAGA

    100        110        120        130        140        150        160        170        180
CAAATCCAAGAAACTGAGGACTACAAATGAGCATTCTCAGACTTGTCATTGGGGTAATCTCCTTCAGGACATTATTCTCCAAGTATTTAAATAT

190        200        210        220        230        240        250        260        270        280
TTGCCTCTTCTTGACCGGGCTCATGCTTCACAAGTTTCCCGCAACTGGAACCAGGTATTTCACATCCCTGACTTGTGCAGATGTTTTGAATTTG

    290        300        310        320        330        340        350        360        370
AACTGAATCAGCCAGCTACATCTTATTTGAAAGCTACCCATCCAGAGCTGATCAAACAGATTATTAAAAGACATTCAAACCATCTACAATATGT

380        390        400        410        420        430        440        450        460        470
CAGCTTCAAGGTGGACAGCAGCAAGGAATCAGCTGAAGCAGCTTGTGATATACTATCGCAACTTGTGAATTGCTCTTTAAAAACACTTGGACTT

    480        490        500        510        520        530        540        550        560
ATTTCAACTGCTCGACCAACGTTTATCGATTTACCAAAGTCTCACTTTATCTCTGCACTGACAGTTGTGTTCGTAAACTCCAAATCCCTGTCTT

    570        580        590        600        610        620        630        640        650
CGCTTAAGATAGATGATACTCCAGTAGATGATCCATCTCTCAAAGTACTAGTGGCCAACAATAGTCATACACTCAAGCTGTTGAAAATGACCAG

660        670        680        690        700        710        720        730        740        750
CTGTCCTCATGTCTCTCCAGCAGGTATCCTTTGTCTGCTGATCAGTGTCACGGCTTAAGAGAACTAGCCCTGAACTACCACTTATTCAGTGAT

    760        770        780        790        800        810        820        830        840
GAGTTGTTACTTCCATTGTCTTCTCAAAAACATGTTCCATTAGAACATTTGCGCCATTGATGTAGTCAGTGAGAATCCTGGACACACACACTTCC

850        860        870        880        890        900        910        920        930        940
ATACTATTCAGAAGAGTAGCTGGGATGCTTTCATCAGACATTCACCCAAAGTGAACTTAGTGATCTATTTTTTTTATATGAAGAAGAATTTGA
```

FIG.5B

```
      950       960       970       980       990      1000      1010      1020      1030
CCCCTTCTTTCCCCTATGAAATACCTCCCACCCATCTGTACTTTGGGAGATCAGTAAGCAAAGATGTGCTTGGCCCGTGTGGGAATGACATGCCCT

     1040      1050      1060      1070      1080      1090      1100      1110      1120
ACACTGGTTGAACTACTATCTGTGCAAATGAATTACAGCCCACTTGATGAAGAGTTAATTCCCATTGCAGAAGGTTGCAAAAATTTGTCAGCTA

     1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
TTGGACTAGGGAAATGTGAAGTCTCATGTACTGCCTTTGTTGAGTTGTGTGAAGATGTGTGGTGGCCCCTATCTCAATTATCCATTATGGAAGA

     1230      1240      1250      1260      1270      1280      1290      1300      1310
AGTACTAATTCCTGACCAAAAGTATAGTTGCACCAGATTCACTGGGAAGTGTCCAACCATCTTGGTACGGTGTGGTTTCCCCACATGATCCCC

     1320      1330      1340      1350      1360      1370      1380      1390      1400
ACTTGGTAAAAACTGCATGATGAATACCACCTTAATTTGCAGCAAATGTATTATAATTAAAGTTTTATTTGCTGTAAAAAAAAAAAAAAA
```

FIG.5C

```
        10         20         30         40         50         60
MKRNSLSVENKIVQLSGAAKQPKVGFYSSLNQTHTHTVLLDWGSLPHHVVLQIFQYLPLL

        70         80         90        100        110        120
DRACASSVCRRWNEVFHISDLWRKFEFELNQSATSSFKSTHPDLIQQIIKKHFAHLQYVS

       130        140        150        160        170        180
FKVDSSAESAEAACQILSQLVNCSIQTLGLISTAKPSFMNVSESHFVSALTVVFINSKSL

       190        200        210        220        230        240
SSIKIEDTPVDDPSLKILVANNSDTLRLPKMSSCPHVSSDGILCVADRCQGLRELALNYY

       250        260        270        280        290        300
ILTDELFLALSSETHVNLEHLRIDVVSENPGQIKFHAVKKHSWDALIKHSPRVNVVMHFF

       310        320        330        340        350        360
LYEEEFETFFKEETPVTHLYFGRSVSKVVLGRVGLNGPRLIELVVCANDLQPLDNELICI

       370        380        390        400        410        420

AEHCTNLTALGLSKCEVSCSAFIRFVRLCERRLTQLSVMEEVLIPQEDYSLDEIHTEVSK

       430
YLGRVWFPDVMPLW
```

FIG.6A

```
          10         20         30         40         50         60
ACATTTCTAATGTTTACAGAATGAAGAGGAACAGTTTATCTGTTGAGAATAAAATTGTCCAGTTGTCA

 70         80         90        100        110        120        130
GGAGCAGCGAAACAGCCAAAAGTTGGGTTCTACTCTTCTCTCAACCAGACTCATACACACACGGTTCTT

140        150        160        170        180        190        200
CTAGACTGGGGGAGTTTGCCTCACCATGTAGTATTACAAATTTTTCAGTATCTTCCTTTACTAGATCGG

 210        220        230        240        250        260        270
GCCTGTGCATCTTCTGTATGTAGGACGTCGAATGAAGTTTTTCATATTTCTGACCTTTGGAGAAAGTTT

 280        290        300        310        320        330        340
GAATTTGAACTGAACCAGTCAGCTACTTCATCTTTTAAGTCCACTCATCCTGATCTCATTCAGCAGATC

 350        360        370        380        390        400        410
ATTAAAAAGCATTTTGCTCATCTTCAGTATGTCAGCTTTAAGGTTGACAGTAGCGCTGAGTCAGCAGAA

 420        430        440        450        460        470        480
GCTGCCTGTGATATACTCTCTCAGCTGGTAAATTGTTCCATCCAGACCTTGGGCTTGATTTCAACAGCC

 490        500        510        520        530        540        550
AAGCCAAGTTTCATGAATGTGTCGGAGTCTCATTTTGTGTCAGCACTTACAGTTGTTTTTATCAACTCA

 560        570        580        590        600        610        620
AAATCATTATCATCAATCAAAATTGAAGATACACCAGTGCATGATCCTTCATTGAAGATTCTTGTGGCC

 630        640        650        660        670        680        690
AATAATAGTGACACTCTAAGACTCCCAAAGATGAGTAGCTGTCCTCATGTTTCATCTGATGGAATTCTT

 700        710        720        730        740        750
TGTGTAGCTGACCGTTGTCAAGGCCTTAGAGAACTGCGCTTGAATTATTACATCCTAACTGATGAACTT

760        770        780        790        800        810        820
TTCCTTGCACTCTCAAGCCAGACTCATGTTAACCTTGAACATCTTCGAATTGATGTTGTGAGTGAAAAT

830        840        850        860        870        880        890
CCTGGACAGATTAAATTTCATGCTCTTAAAAAACACAGTTGGGATGCACTTATTAAACATTCCCCTAGA

900        910        920        930        940        950        960
GTTAATGTTGTTATGCACTTCTTTCTATATGAAGAGGAATTCGACACGTTCTTCAAAGAAGAAACCCCT
```

FIG.6B

970 980 990 1000 1010 1020 1030
GTTACTCACCTTTATTTTGGTCGTTCAGTCAGCAAAGTGGTTTTACGACGGGTACGTCTCAACTGTCCT

1040 1050 1060 1070 1080 1090 1100
CGACTGATTGAGTTAGTGGTGTGTGCTAATGATCTTCAGCCTCTTGATAATGAACTTATTTGTATTGCT

1110 1120 1130 1140 1150 1160 1170
GAACACTGTACAAACCTAACAGCCTTGGGCCTCAGCAAATGTGAAGTTAGCTGCAGTGCCTTCATCAGG

1180 1190 1200 1210 1220 1230 1240
TTTGTAAGACTGTGTGAGAGAAGGTTAACACAGCTCTCTGTAATGGACGAAGTTTTGATCCCTGATGAG

1250 1260 1270 1280 1290 1300 1310
GATTATAGCCTAGATGAAATTCACACTGAAGTCTCCAAATACCTGGGAAGAGTATGGTTCCCTGATGTG

1230
ATGCCTCTCTGC

FIG.6C

```
        10        20        30        40        50        60
MAGSEPRSGTNSPPPPFSDWGRLEAAILSGWKTFWQSVSKDRVARTTSREEVDEAASTLT

        70        80        90       100       110       120
RLPIQVQLYILSFLSPHDLCQLGSTNHYWNETVRNPILWRYFLLRDLPSWSSVDWKSLPY

       130       140       150       160       170       180
LQILKKPISEVSDGAFFDYMAVYLMCCPYTRRASKSSRPMYGAVTSFLHSLIIPNEPRFA

       190       200       210       220       230       240
LFGPRLEQLNTSLVLSLLSSEELCPTAGLPQRQIDGIGSGVNFQLNNQHKFNILILYSTT

       250       260       270       280       290       300
RKERDRAREEHTSAVNKMFSRHNEGQDRPQSRYSVIPQIQKLCEVVDGFIYVANAEAHKR

       310       320       330       340       350       360
HEWQDEFSHIMAMTDPAFGSSGRPLLVLSCISQGDVKRMPCFYLAHELHLNLLNHPWLVQ

       370       380       390       400       410       420
DTEAETLTGFLNGIEWILEEVESKRAR*FSFQILGTETI*NLLLRS*CEYLLSQPTLSCL

       430       440       450       460       470       480
FADRLSFGQL*LLCFLYYFYFLP*INYKKRVSVLVFSPKMNL*TFFW*FLYFLSF*KY*I

L
```

FIG.7A

```
          10         20         30         40         50         60
ATGCCCGGAAGCGAGCGCGCGCAGCGGAACAAATTCGCCGCCCCCGCCCTTCAGCGACTGGGCCCCCTG

 70         80         90        100        110        120        130
GAGCCGCCATCCTCAGCGGCTCGAAGACCTTCTGCCAGTCAGTCAGCAAGGATAGGGTGCCCCTACC

140        150        160        170        180        190        200
ACCTCCCGGACGAGGTGCATCAGGCGGCCAGCACCCTGACCGCGCTGCCGATTCATGTACAGCTATAT

210        220        230        240        250        260        270
ATTTTGTCCTTTCTTTCACCTCATGATCTGTCTCAGTTGCGAAGTACAAATCATTATTCGAATGAAACT

280        290        300        310        320        330        340
GTAACAAATCCAATTCTGTGCAGATACTTTTTGTTCAGGGATCTTCCTTCTTGCTCTTCTGTTGACTGC

350        360        370        380        390        400        410
AAGTCTCTTCCATATCTACAAATCTTAAAAAAGCCTATATCTCAGGTCTCTCATCGCTGCATTTTTTGAC

420        430        440        450        460        470        480
TACATGCCAGTCTATCTAATGTCCTGTCCATACACAACAACACCTTCAAAATCCAGCCGTCCTATCTAT

490        500        510        520        530        540        550
GCAGCTCTCACTTCTTTTTTACACTCCCTGATCATTCCCAATCAACCTCGATTTCCTCTCTTTGGACCA

560        570        580        590        600        610        620
CGTTTCGAACAATTCAATACCTCTTTGGTGTTCASCTTGCTGTCTTCAGACGAACTTTCCCCAACAGCT

630        640        650        660        670        680        690
CGTTTGCCTCAGAGCCAGATTGATGGTATTGGATCAGGAGTCAATTTTCAGTTGAACAACCAACATAAA

700        710        720        730        740        750
TTCAACATTCTAATCTTATATTCAACTACCAGAAAGCAAAGACATAGAGCAAGGGAAGAGCATACAAGT

760        770        780        790        800        810        820
GCAGTTAACAACATGTTCAGTCCACACAATCAACGTGATGATCGACCAGGAAGCCCGTACAGTGTGATT

830        840        850        860        870        880        890
CCACAGATTCAAAAACTGTGTGAAGTTGTAGATGGGTTCATCTATGTTGCAAATGCTGAAGCTCATAAA

900        910        920        930        940        950        960
AGACATGAATGCCAAGATGAATTTTCTCATATTATGCCAATGACAGATCCAGCCTTTGGGTCTTCGCCA
```

FIG.7B

970 980 990 1000 1010 1020 1030
AGACCATTGTTGGTTTTATCTTGTATTTCTCAAGGGGATGTAAAAAGAATGCCCTGTTTTTATTTGGCT

1040 1050 1060 1070 1080 1090 1100
CATGAGCTGCATCTGAATCTTCTAAATCACCCATGGCTGGTCCAGGATACAGAGGCTGAAACTCTGACT

1110 1120 1130 1140 1150 1160 1170
GGTTTTTTGAATGGCATTGAGTGGATTCTTGAAGAAGTGGAATCTAAGCGTGCAAGATGATTCTCTTTT

1180 1190 1200 1210 1220 1230 1240
CAGATCTTGGGAACTGAAACCATTTGAAATTTATTACTAAGGTCGTGATGTGAATATTTGCTCAGTCAG

1250 1260 1270 1280 1290 1300 1310
CCCACCTTGTCCTGCCTTTTTGCAGATAGGCTTTCATTTGGACAGCTATAACTGCTGTGTTTTTTATAT

1320 1330 1340 1350 1360 1370 1380
TATTTTTACTTTTTACCATAAATCAATTACAAGAAAAGAGTTTCAGTCCTAGTATTTAGCCCCAAAATG

1390 1400 1410 1420 1430 1440
AACCTTTAAACATTTTTTTGGTAATTTTTATATTTTCTGTCTTTTTAAAAATATTAAATTTTGC

FIG.7C

```
        10         20         30         40         50         60
MSRRPCSCALRPPRCSCSASPSAVTAAGRPRPSDSCKEESSTLSVKMKCDFNCNHVHSGL

        70         80         90        100        110        120
KLVKPDDIGRLVSYTPAYLEGSCKDCIKDYERLSCIGSPIVSPRIVQLETESKRLHNKEN

       130        140        150        160        170        180
QHVQQTLNSTNEIEALETSRLYEDSGYSSFSLQSGLSEHEEGSLLEENFGDSLQSCLLQI

       190        200        210        220        230        240
QSPDQYPNKNLLPVLHFEKVVCSTLKKNAKRNPKVDREMLKEIIARGNFRLQNIIGRKMG

       250        260        270        280        290        300
LECVDILSELFRRGLRHVLATILAQLSDMDLINVSKVSTTWKKILEDDKGAFQLYSKAIQ

       310        320        330        340        350        360
RVTENNNKFSPHASTREYVMFRTPLASVQKSAAQTSLKKDAQTKLSNQGDQKGSTYSRHN

       370        380        390        400        410        420
EFSEVAKTLKKNESLKACIRCNSPAKYDCYLQRATCKREGCGFDYCTKCLCNYHTTKDCS

       430        440
DGKLLKASCKIGPLPGTKKSKKNLRRL
```

FIG.8A

```
                10                  20                  30                  40                  50                  60                  70                  80                  90
AGGTTGCTCAGCTCCCCCCGGAGCGGTTCCTCCACCTGAGGACAGACACCACCTCGGTTGGCATGAGCCGGCCCCTGCACCTCCGCCCCTACCG

           100                 110                 120                 130                 140                 150                 160                 170                 180
CCACCCCCGCTGCTCCTGCAGCGGCCAGCCCCAGCCGACAGCGGCCGGGCGGCCCTGGACCCTCGGATAGTTGTAAGAGAAAGTTCTACCC

           190                 200                 210                 220                 230                 240                 250                 260                 270                 280
TTTCTGTCAAAATGAAGTGTGATTTTAATTGTAACCATGTTCATTCCGGACTTAAACTGGTAAAACCTGATGACATTGGAAGACTAGTTTCCTA

           290                 300                 310                 320                 330                 340                 350                 360                 370
CACCCCTGCATATCTGGAAGGTTCCTGTAAAGACTGCATTAAAGACTATGAAAGGCTGTCATGTAATTGGGTCACCGATTGTGACCCCTAGGATT

           390                 400                 410                 420                 430                 440                 450                 460                 470
GTACAACTTGAAACTGAAAGCAAGCGCTGCATAAGAAGAAATCAACATGTGCAACACACACTTAATAGTACAAATGAAATTAGAAGCACTAG

           480                 490                 500                 510                 520                 530                 540                 550                 560
ACACCAGTAGACTTTATGAAGCACTGCTGCCTATTCCTCATTTTTCTCTACAAAGTGCCCTCAGTGACATGAGAAGGTTGCCTCCTGCAGCAAA

           570                 580                 590                 600                 610                 620                 630                 640                 650
TTTCCGTCACAGTCTACAATCCTCCCTGCTACAAATACAAAGCCCACCAATATCCCAACAAAAACTTGCTCCCAGTCTCTTCATTTTGAAAAA

           660                 670                 680                 690                 700                 710                 720                 730                 740                 750
GTGCTTTGTTCCAACATTAAAAAAGAATCCAAAAGGAAATCCTAAGTAGATCCGGAGATGCTGAAGGAAATTATACCCAGAGGAAATTTTAGAC

           760                 770                 780                 790                 800                 810                 820                 830                 840
TGCAGAATATAATTGGCAGAAAATGGGCCTAGAATGTGTAGATATTCTCAGCCAACTCTTTTCGAAGGGCACTCAGACATGTCTTAGCAACTAT

           850                 860                 870                 880                 890                 900                 910                 920                 930                 940
TTTAGCAGACTCAGTCACATGGACTTAATCAATGTGTCTAAAGTAGGCACAAGTGGAAGAGATGCTATGAAGATGTGATAAGGGCCATTCCAG
```

FIG.8B

950 960 970 980 990 1000 1010 1020 1030
TTGTACAGTAAACCAATACAAAGAGTTACCCAAAACAACAATAAATTTTCACCTCATCCTCAACCAGACAATATGTTATGTTCAGAACCCCAC

1040 1050 1060 1070 1080 1090 1100 1110 1120
TGCCTTCTGTTCAGAAATCAGCACCCCAGACTTCTCTCAAAAAAGATGCTCAAACCAAGTTATCCAATCAAGGTGATCAGAAACGTTCTACTTA

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
TAGTCGCACACAATGAATTCTCTGAGGTTGCCAAGACATTCAAAAAGAACGAAAGCCTCAAACCCTGTATTCGCTGTAATTCACCTGCAAAATAT

1230 1240 1250 1260 1270 1280 1290 1300 1310
GATTGCTATTTACAACGGCCAACCTGCAAACGACAAGCCTGTGGATTGATTATTGTACGAAGTGTCTCTGTAATTATCATACTACTAAAGACT

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
GTTCAGATGGCAAGCTCCTCAAAGCCAGTTGTAAAATAGGTCCCCTGCCTGGTACAAAGAAAAGCAAAAAGAATTTACGAAGATTGTGATCTCT

1420 1430 1440 1450 1460 1470 1480 1490 1500
TATTAAATCAATTGTTACTGATCATGAATGTTAGTTTAACTTAAAAAAAATTGTATTGTGATTTTCAATTTTATGTTG

1510 1520 1530 1540 1550 1560 1570 1580 1590
AAATCGGTGTAGTATCCTCAGGTTTTTTTCCCCCCACAAGATAAAGAGGATAGACAACCTCTTAAAATATTTTTACAATTTAATGACAAAAAGT

1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
TTAAAATTCTCAATACAAATCAAACAATTTAAATATTTTAAGAAAAAAGGAAAACTACATAGTGATACTCAGCGTAAAAAAAAAATTCATTCAA

1700 1710 1720 1730 1740 1750 1760 1770 1780
ITTTATGGTAAAGCAAACCCATGCAATTTTACCTACACAGTCTTAAATATGTCTGGTTTCCATCTGTTACCATTTCAGCACATTTATGTTCCT

1790 1800 1810 1820 1830 1840 1850 1860 1870 1880
CTTACTCAATTGATACCAACAGAAATATCAACTTCTGGAGTCTATTAAATGTCTTGTCACCTTTCTAAACCTTTTTTCATTGTCTGTATTTCC

1890 1900 1910 1920 1930 1940 1950 1960 1970
CAACAAGTATCCTTTGTAAAACTTCCTTGTTTTCCTTATTTCTGAAATCTGTTTTAATATTTTTGTATACATGTAAATATTTCTGTATTTTT

1980 1990 2000 2010 2020 2030 2040 2050 2060
TATATGTCAAAGAATATGTCTCTTGTATGTACATATAAAAATAAATTTTGCTCAATAAAATTGTAAGCTTAAAAAAAAAAAAAAAAACTCGAG

2070
ACTAGTGC

FIG.8C

```
        10         20         30         40         50         60
ARSGASALRRRRVQVWVLSRPPPGGGDSFRTRRPQRGPGPGGSQAMDAPHSKAALDSINE

        70         80         90        100        110        120
LPDNILLELFTHVPARQLLNCRLVCSLWRDLIDLLTLWKRKCLRKGFITKDWDQPVADW

       130        140        150        160        170        180
KIFYFLRSLHRNLLRNPCAENDMFAWQIDFNGGDRWKVDSLPGAHGTEFPDPKVKKSFVT

       190        200        210        220        230        240
SYELCLKWELVDLLADRYWEELLDTFRPDIVVKDWFAARADCGCTYQLKVQLASADYFVL

       250        260        270        280        290        300
ASFEPPPVTIQQWNNATWTEVSYTFSDYPRGVRYILFQHGGRDTQYWAGWYGPRVTNSSI

       310        320        330
VVSPKMTRNQASSEAQPGQKHGQEEAAQSPYGAVVQIF
```

FIG.9A

```
          10         20         30         40         50         60         70         80         90
GCGCGTTCGGGAGCTTCGGCCCTGCGTAGGAGGCGGGTGCACGTGTGGGTGCTGAGCCCCCCCCCCCCTGGAGGGGGAGACAGCTTCAGGACAC

     100        110        120        130        140        150        160        170        180
GCAGGCCGCACGGAGGGCCCGGGCCCGGGGGATCCCAGGCCATGGACGCTCCCCACTCCAAAGCAGCCCTGGACAGCATTAACGAGCTGCCCGA

190        200        210        220        230        240        250        260        270        280
TAACATCCTGCTGGAGCTGTTCACGCACGTGCCCCGCCCGCCAGCTGCTGCTGAACTGCCGCCCTGGTCTGCAGCCTCTGGCGGGACCTCATCGAC

     290        300        310        320        330        340        350        360        370
CTCCTGACCCTCTGGAAACGCAAGTGCCTGCGAAAGGGCTTCATCACCAAGGACTGGGACCAGCCCGTGGCCGACTGCAAAATCTTCTACTTCC

380        390        400        410        420        430        440        450        460        470
TACGGAGCCTGCATAGGAACCTCCTGCCCAACCCGTGTGCTGAAAACGATATGTTTGCATGGCAAATTGATTTCAATGGTGCGGCACCGCTGGAA

     480        490        500        510        520        530        540        550        560
GGTGGATAGCCTCCCTGGAGCCCACGGCACAGAATTTCCTGACCCCAAACTCAAGAAGTCTTTTGTCACATCCTACCAACTGTGCCTCAAGTGG

570        580        590        600        610        620        630        640        650
GAGCTGGTGGACCTTCTAGCCGACCGCTACTGCGAGGAGCTACTAGACACATTCCGCCCGGACATCGTGGTTAAGGACTGGTTTGCTGCCAGAG

660        670        680        690        700        710        720        730        740        750
CCGACTGTGGCTGCACCTACCAACTCAAAGTGCAGCTGGCCTCGGCTGACTACTTCGTGTTGGCCTCCTTCGAGCCCCCACCTGTGACCATCCA

     760        770        780        790        800        810        820        830        840
ACAGTGGAACAATGCCACATCGACACAGGTCTCCTACACCTTCTCAGACTACCCCCGGGGTGTCCGCTACATCCTCTTCCAGCATGGGGGCAGG

850        860        870        880        890        900        910        920        930        940
GACACCCAGTACTGGGCAGGCTGGTATGGGCCCCGAGTCACCAACAGCAGCATTGTCGTCAGCCCCAAGATGACCAGGAACCAGGCCTCGTCCG
```

FIG.9B

```
       950       960       970       980       990       1000      1010      1020      1030
AGGCTCAGCCTGGGCAGAAGCATGGACAGGAGGAGGCTGCCCAATCGCCCTACGGAGCTGTTGTCCAGATTTTCTGACAGCTGTCCATCCTGTG

    1040      1050      1060      1070      1080      1090      1100      1110      1120
TCTGGGTCAGCCAGAGGTTCCTCCAGGCAGGAGCTGAGCATGGGGTGGGCAGTGAGGTCCCTGTACCAGCGACTCCTGCCCCGGTTCAACCCTA

1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
CCAGCTTGTGGTAACTTACTGTCACATAGCTCTGACGTTTTGTTGTAATAAATGTTTTCAGGCCGGGCACTGTGGCTCACGCCTGTAATCCCAG

      1230      1240      1250      1260      1270      1280      1290      1300      1310
CACTTTGGGAGACCGAGGCAGGTGGATCACGAGGTCAGGAGACAGAGACCATCCTGGCCAACACGGTGAAACCCTGTCTCTACTAAAAATACAA

1320      1330      1340      1350      1360      1370      1380      1390      1400      1410
AAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGATGCAGAAGAATGGCGTGAACCCGGAAGGCAGAGCTTGC

       1420      1430      1440      1450      1460      1470      1480      1490      1500
AGTGAGCCGAGATCACGCCACTGCACTCCAGCCTGGGTGACAGAGCGAGACTCTGGCTCATAAAATAATAATAATAATAAATAAATAAAAAATA

  1510      1520      1530
AATGGTTTTCAGTAAAAAAAAAAAAAAAAAA
```

FIG.9C

```
        10         20         30         40         50         60
MSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILHDDIPPPNIPSSTDSEHSSLQN

        70         80         90        100        110        120
NEQPSLATSSNQTSIQDEQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQQNAHMAEG

       130        140        150        160        170        180
TGFYPSEPLLCSESVEGQVFHSLETLYQSADCSDANDALIVLIHLLMLESGYIPQGTEAK

       190        200        210        220        230        240
ALSLPEKWKLSGVYKLQYMHHLCEGSSATLTCVPLGNLIVVNATLKINNEIRSVKRLQLL

       250        260        270        280        290        300
PESFICKEKLGENVANIYKDLQKLSRLFKDQLVYPLLAFTRQALNLPNVFGLVVLPLELK

       310        320        330        340        350        360
LRIFRLLDVRSVLSLSAVCRDLFTASNDPLLWRFLYLRDFRDNTVRVQDTDWKELYRKRH

       370        380        390        400        410        420
IQRKESPKGRFVLLLPSSTHTIPFYPNPLHPRPFPSSRLPPGIGGEYDQRPTLPYVGDP

       430        440        450        460        470        480
ISSLIPGPGETPSQLPPLRPRFDPVGPLPGPNPILPGRGGPNDRFPFRPSRGRPTDGRLS

FM
```

FIG.10A

```
10                  20                  30                  40                  50                  60                  70                  80                  90
TGGAAATTCCCATGGACCATGTCTAATACCCGATTTACAATTGATGACTACAAGGATCCCCTCACTGGACATGAAGAAGACCTTGGCTTCATA

100                 110                 120                 130                 140                 150                 160                 170                 180
TCGCCATTCGTTTCTGGGACTTGATATGTTTGATTCTTCACGATGACATTCCCACCGCCTAATATTACCTTCATCCACAGATTCCAGAGCATTCTTCA

190                 200                 210                 220                 230                 240                 250                 260                 270                 280
CTCCAGACAATGAGCAACCCTCTTTGGCCACCAGCTCCAATCAGACTACGCAATACAGGATGAACACCCAAGTGATTCATTCCAAGGACACGCCAG

290                 300                 310                 320                 330                 340                 350                 360                 370
CCCAGTCTGGTGTTTGGAATGACGACACTATGTTAGGGCCTAGTCAAAAATTTTGAACCTGAGTCAATTCAACATAATGCGCATATGGCAGACGG

380                 390                 400                 410                 420                 430                 440                 450                 460                 470
CACACGTTTCTATCCCTCAGAACCCTGCTCTGTAGTGAATCGGTGGAAGGCCAAGTGCCACATTCATTAGAGACCTTGTATCAATCAGCTCAC

480                 490                 500                 510                 520                 530                 540                 550                 560
TGTTCTGATGCCAATGATGCGTTGATAGTGTTGAATACATCTTCTCATGTTGGAGTCAGGTTACATACCTCAGGCCACCGAAGCCAAAGCCACTGT

570                 580                 590                 600                 610                 620                 630                 640                 650
CCCTCCCGCAGAAGTTGCAGCGGGTGCTATAAGCTGCAGTACATCCATCTCTGCCAGGGCAGCTCGCCTACTCTCACCTGTGTGCC

660                 670                 680                 690                 700                 710                 720                 730                 740                 750
TTTGGCAAACCTGATTGTTGTAAATCCTACACTAAAAATCAACAATGAGATTCAAAGTGTGAAAAGATTCCAGCTGCTACCAGAATCTTTTATT

760                 770                 780                 790                 800                 810                 820                 830                 840
TGCAAGGAGAAACTAGGGGAAAATGTACCCAACATATACAAAGATCTTCTCGGCTCTTTAAAGACCAGCTGGTGTATCCTCTTC

850                 860                 870                 880                 890                 900                 910                 920                 930                 940
TGGCTTTTACCCCACAAGCACTCAACCTACCAAATGTATTTGGGTTGGTCCTCCACTTGGAACTACGGATCTTCCGCACTTCTGGA
```

FIG.10B

950 960 970 980 990 1000 1010 1020 1030
TGTTCGTTCCGTCTTGTCTTTGTCTGCCGTTTGTCCTGACCTCTTTACTGCTTCAAATGACCCACTCCTGTGGAGGTTTTTATATCTGCGTGAT

1040 1050 1060 1070 1080 1090 1100 1110 1120
TTTCGAGACAATACTGTCAGAGTTCAAGACACAGATTGGAAAGAACTGTACAGGAAGAGGCACATACAAAGAAAACAATCCCCGAAAGGCCGGT

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
TTGTGCTGCTCCTGCCATCGTCAACCCACACCATTCCATTCTATCCCAACCCCTTGCACCCTAGCCCATTTCCTAGCTCCCCCCTTCCTCCAGG

1230 1240 1250 1260 1270 1280 1290 1300 1310
AATTATCGGGGGTGAATATGACCAAAGACCAACACTTCCCTATGTTCGAGACCCAATCAGTTCACTCATTCCTGGTCCTGGGGAGACGCCCAGC

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
CAGTTACCTCCACTGAGACCACGCTTTGATCCAGTTGGCCCACTTCCAGGACCTAACCCCATCTTGCCAGGGCGAGGCGGCCCCAATGACAGAT

1420 1430 1440 1450 1460 1470 1480 1490 1500
TTCCCTTTAGACCCAGCAGGGGTCGGCCAACTGATGGCCGCCTGTCATTCATGTGATTGATTTGTAATTTCATTTCTGGAGCTCCATTTGTTTT

1510 1520 1530 1540 1550 1560 1570 1580 1590
TGTTTCTAAACTACAGATGTCACTCCTTGGGGTGCTGATCTCGAGTGTTATTTTCTGATTGTGGTGTTGAGAGTTGCACTCCCAGAAACCTTTT

1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
AAGAGATACATTTATAGCCCTAGCGGTCGTATGACCCAAAGGTTCCTCTGTGACAAGGTTGGCCTTGGGAATAGTTGGCTGCCAATCTCCCTGC

1700 1710 1720 1730 1740 1750 1760
TCTTGGTTCTCCTCTAGATTGAAGTTTGTTTTCTGATGCTGTTCTTACCAGATTAAAAAAAAGTGTAAATT

FIG.10C

```
        10         20         30         40         50         60
ETSKLG*SAVLAPAAGGTLSSEGRSAVSGILIAVTSTGVDK*SLNQLLHGLGTSSRLSHF

        70         80         90        100        110        120
PFG*KSPPRGQFVAAAVEIAGRSGLQMGQGLWRVVRNQQLQQEGYSEQGYLTREQSRRMA

       130        140        150        160        170        180
ASNISNTNHRKQVQGGIDIYHLLKARKSKEQEGFINLEMLPPELSFTILSYLNATDLCLA

       190        200        210        220        230        240
SCVWQDLANDELLWQGLCKSTWGHCSIYNKNPPLGFSFRKXYMQLDEGSLTFNANPDEGV

       250        260        270        280        290        300
NYFMSKGILDDSPKEIAKFIFCTRTLNWKKLRIYLDERRDVLDDLVTLHNFRNQFLPNAL

       310        320        330        340        350        360
REFFRHIHAPEERGEYLETLITKFSHRFCAGNPDLMRELGLSPDAVYVLCYSLILLSIDL

       370        380        390        400        410        420
TSPHVKNKMSKREFIRNTRRAAQNISEDFVGHLYDNIYLIGHVAA*KAQLLGLQFLLQTK

       430        440        450        460        470        480
ATQGLSRYGGYISAGHCSLSIQSSFSVQPFFLLPFSILVISLGN*IILQNFS*FCLSRFA

       490        500        510        520        530        540
QSRATV*HSC*RMIN*HYTLKDGVFVH*ICLKNFIHFHSLYKYHVMCTYLTKEIYSHNYF

       550        560        570        580        590        600
IVKILTKVFPFLSN*VLKFI*F*SETIVXVKVRSDFRQKPIPASFSFKL*RVLICYYITM

       610        620        630        640        650
QNWQLFL*YKFII*FFILKTGLIKSR*VL*TI*DF*NIKIYDLHS*E*NKIXLELW
```

FIG.11A

```
                10        20        30        40        50        60        70        80        90
CGAAACGTCAAAATTCGGATAGTCCGCCACTTCTGCCCCCCTGCAGCTTACCCTGATTCTGACGGTCGTACGTGCTGTTCTGCGTATTCTC

               100       110       120       130       140       150       160       170       180
ATCCCGGTCACCTCTACCGGTGTGGACAAGTAAAGTTGAATCAGCTTCTCCATGGCCTCGGCCACCAGTTCCCCGCTCAGCCATTTTCCTTTTC

               190       200       210       220       230       240       250       260       270       280
CCTTAAAGTCCCCCCCCCAGACCCAATTCGTCCGCCGCCGCTGGACATCCCAGCTTCCACGCTTGCAGATGGGTCAACCGTTGTGGAGAGT

               290       300       310       320       330       340       350       360       370
CGTCAGAAACCAGCAGCTGCAACAAGCAAGCCTACCTCAGCAGAGAGCAGAGCAGGAGAATGCGCTGCCAGCAACATTCT

               380       390       400       410       420       430       440       450       460       470
AACACCAATCATCGTAAACAAGTCCAAGCAGCACATTGCACATATATCATCTTTTGAAGGCAAGCAAATCCAAACAGCAACGCAAGCATTCATTAATT

               480       490       500       510       520       530       540       550       560
TGGAAATGTTGCCCTCCTGAGCTTAAGCTTTACCATCTTGTCCTACCTGAATGCAACTGACCTTTGCCTTCATGTGTTTGCCAGCACCTTGC

               570       580       590       600       610       620       630       640       650
CAATCATGACTTCTCTGGCAAGCGTGTGCAAATCCACTTGCCGTCACTGTTCCATATAACAATACAGACCACCTTTAGCATTTTCTTTTAGA

               660       670       680       690       700       710       720       730       740       750
AAAKTGTATATGCACCTGGATCAACCCACCTCACCTTTAATGCCAACCCACGATGCAGCAGTCAACTACTTATGTCCAACGGTATCCTGGATG

               760       770       780       790       800       810       820       830       840
ATTGCCCAAGGAAATAGCAAAGTTATCTCTGTCACAGAACACTCTAAATTGCAAAAACTGACAATCTATCTGATGAAGCAGACATGTCTT

               850       860       870       880       890       900       910       920       930       940
CGATGCACCTTGTAACATTGCATAATTTTACAAATCAGTTCTGCCAAATGCACTGACAGACAATTTTTTGCTATCCATGCCCCTGAACAGCGT
```

FIG.11B

950 960 970 980 990 1000 1010 1020 1030
GCACAGTATCTTGAAACTCTATAACAAAGTTCCTCACATACATTCTGCTTGCCTGCAACCCTGATTTAATGCCAGACAACTTGCCCCTTAGTCCTGATG

1040 1050 1060 1070 1080 1090 1100 1110 1120
CTGCTCATAGTACTGTGCCTACTCTTTCATTCCAATTGACCTCACTAGCCCCTCATGTGAACAATAAAATGTCAAAAAGCGGAATTTAATTGC

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
AAATACCCGTCGCCCTGCTCAAAATATTACTGAACAATTTTGTAGCCCATCTTTAATACAATATCTACGTTATTGCCCATGTGCCTGCATAAAAA

1230 1240 1250 1260 1270 1280 1290 1300 1310
GCACAATTGCTACGAACTTCACTTTTTACTTCAGACTAAAGCTACCCAAGAACTTAGCAGATAATGCGGCGTTACATCACGTCGTCATTGTAGCCC

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
TGAGTATACAAATCAACCTTCACTGTGCAACCTTTTTTTTCTTTCCCATTTTCTATTTTAGTAATTTCCTTGCGGCAACTAAATAATTTTGCAGAA

1420 1430 1440 1450 1460 1470 1480 1490 1500
TTTTTCCTAATTTTGTTATCAGGTTTTGCACAAAGCAGCCACTGTCTAACGACACCTGTTAACGATGATAAACTGACATTATACTCTAAAA

1510 1520 1530 1540 1550 1560 1570 1580 1590
GATGCTGTATTTGTGCATTAGATTTGCCTGAAAAACTTTATCCATTTCGTTATACAAATACCATGTAATGTGTACATATTTAACTAAAG

1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
AGATTTATAGTCATAATTATTTATGTAAAGATTTTAACTAAAGTTTTTCGTTTTCTCATAAACTGACTTCTGAAATTTATTGATTCTGATC

FIG.11C

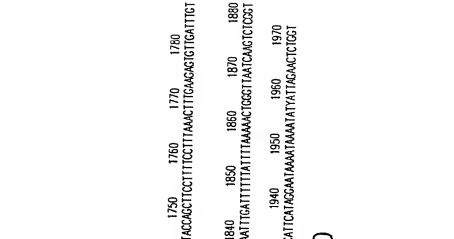
1700 1710 1720 1730 1740 1750 1760 1770 1780
TGAAACTATTGTCTCTCGTAAAATTTAGATCTGACTTGAGRCAGAAACCAATAGCAGCTCCTTTCCTTTAAACTTTGAACAGTGTTGATTTGT
1790 1800 1810 1820 1830 1840 1850 1860 1870 1880
TACTATATTACTATGCAAAACTCCCAGTTATTTTTATATATAAATTTATAATTGATTTTTTTATTTTTAAAAACTGGGTTAATCAAGTCTCCGCT
1890 1900 1910 1920 1930 1940 1950 1960 1970
AAGTCCTTTAAACCAATTTAGGATTTTTAAAACATCAAAATTTATGATTTACATTCATAGGAATAAAATAAAATATTATTGAAACTCTCGCT

FIG.11D

```
        10         20         30         40         50         60
MAAAAVDSAMEVVPALAEEAAPEVAGLSCLVNLPGEVLEYILCCGSLTAADIGRVSSTCR

        70         80         90        100        110        120
RLRELCQSSGKVWKECFRVRWPSLMKHYSPTDYVNWLEEYKVRQKAGLEARKIVASFSKR

       130        140        150        160        170        180
FFSEHVPCNGFSDIENLEGPEIFFEDELVCILNMEGRKALTWKYYAKKILYYLRQQKILN

       190        200        210        220        230        240
NLKAFLQQPDDYESYLEGAVYIDQYCNPLSDISLKDIQAQIDSIVELVCKTLRGINSRHP

       250        260        270        280        290        300
SLAFKAGESSMIMEIELQSQVLDAMNYVLYDQLKFKGNRMDYYNALNLYMHQVLIRRTGI

       310        320        330        340        350        360
PISMSLLYLTIARQLGVPLEPVNFPSHFLLRWCQGAEGATLDIFDYIYIDAFGKGKQLTV

       370        380        390        400        410        420
KECEYLIGQHVTAALYGVVNVKKVLQRMVGNLLSLGKREGIDQSYQLLRDSLDLYLAMYP

       430        440        450        460        470        480
DQVQLLLLQARLYFHLGIWPEKVLDILQHICTLDPGQHGAVGYLVQHTLEHIERKKEEVC

       490        500        510        520        530        540
VEVKLRSDEKHRDVCYSIGLIMKHKRYGYNCVIYGWDPTCMMCHEWIRMMVHSLPHGHH

       550        560        570        580        590        600
QPFYNVLVEDGSCRYAAQENLEYNVEPQEISHPDVGRYFSEFTGTHYIPNAELEIRYPED

       610        620
LEFVYETVQNIYSAKKENIDE
```

FIG.12A

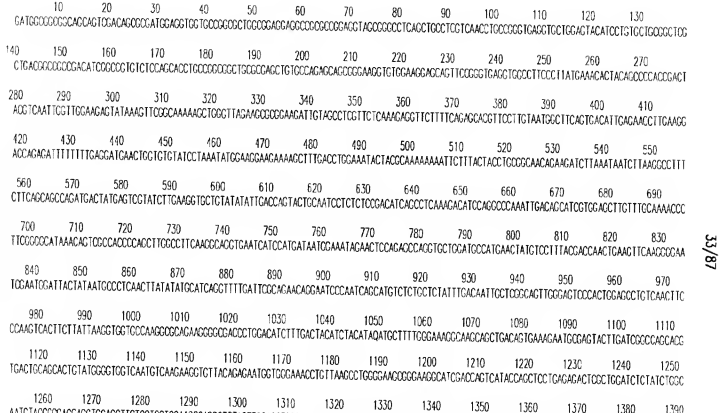
10 20 30 40 50 60 70 80 90 100 110 120 130
GATGCCCCCCGCCACCAGTCCACAGCCCCATGCAGGTGCTCCCGCCCTCCCGCAGCAGCCCCCGCCCGCAGCTAGCCGCCCTCACCTCCCTCGTCAACCTGCCCCGTCAGCTCCTGCACTACATCCTGTCCTGCCCCTCG
140 150 160 170 180 190 200 210 220 230 240 250 260 270
CTCACGCCCCCCGACATCCGCCCTGTCTCCAGCACCTCCCCCCCCGCTGCCCAGCTGTCCCAGAGCACCCGGAAGGTGTGCAAGGACACAGTTCCGCGTCAGCTCCCTTCCCTATCAACACTACAGCCCCACCGACT
280 290 300 310 320 330 340 350 360 370 380 390 400 410
ACGTCAATTCGTTGCAAGACTATAAAGTTCCGCAAAAAGCTGGCTTAGAAGCCCCGAAGATTGTAGCCTCGTTCTCAAAGACGGTTCTTTTCAGAGCAGCTTCCTGTAATGGCTTCAGTCACATTGAGAACCTTCAAGG
420 430 440 450 460 470 480 490 500 510 520 530 540 550
ACCAGACATTTTTTTTGACGATCAACTGCTGTCTATCCTAAATATGCAAGCAACAAAACCTTTGACCTGCAAATACTACCCAAAAAAAATTCTTTACTACCTCCCGCAACAGATCTTAAATAATCTTAAGCCCTTT
560 570 580 590 600 610 620 630 640 650 660 670 680 690
CTTCAGCAGCCAGATGACTATGAGTCCTATCTTGAAGGTGCTCTATATATTGACCAGTACTCCAATCCTCTCTCCCACATCACCCTCAAACACATCCAGCCCCAAATTGACACCATCGTGCAGCTTGTTTCCAAAACCC
700 710 720 730 740 750 760 770 780 790 800 810 820 830
TTCGGCCCATAAACAGTCGCCACCCCACCTTCCCCTTCAAGCCAGCTGAATCATCCATGATAATCCAAATACAACTCCAGACCCACGTGCTCGATCCCATCAACTATCTCCTTTACCACCAACTCAACTTCAACCCCAA
840 850 860 870 880 890 900 910 920 930 940 950 960 970
TCGAATCGATTACTATAATCCCCTCAACTTATATATGCATCAGCTTTTCATTCCCACAACAGGAATCCCAATCAGCATGTCTCTCCTCTATTTCACAATTGCTCCGCAGTTGCGAGTCCCACTGCAGCCTGTCAACTTC
980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 1110
CCAAGTCACTTCTTATTAACGTGGTCCCAAGGCGCACACGGCGCACCCTCGACATCTTTGACTACATCTACATAQATGCTTTCGGAAACCCAAGCAGCTGACAGTGAAAGAATGCGAGTACTTGATCGCCCACCACG
1120 1130 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250
TGACTGCAGCCACTGTATGGCGTGCTCAATGTCAAGAAGCTGTTACAGAGAATTGGTGCGAAACCTGTTAAGCCTGGCGAACGCGGCAAGGCATCGACCAGTCATACCAGCTCCTGCAGAGACTCGCTGCATCTCTATCTCGC
1260 1270 1280 1290 1300 1310 1320 1330 1340 1350 1360 1370 1380 1390
AATCTACCCGCAGCAGGTCCAGCTTCTCCTCCTCCAAGCCAGCGTTACTTCCACCTGCGAATCTGCCAGAGAAGGTGCTTGACATCCTCCAGCACATCCAAACCCTACACCCGGCGCCACCAGCGCCGCTGCGCTAC

FIG.12B

1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520
CTGCTGCACCACACTCTAGACGCACATCCAGCGCAAAAGCGACGAGCTGGGCCTAGAGGTGAAGCTGCGCTCCGATGAGAGCACACAGATGTCTGCTACTCCATGGGGCTCATTATGAAGCATAAGAGGTATGGCTATA

1530 1540 1550 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660
ACTGTGTGATCTACCGCTGCGACCCCACCTGCATGATGGCACACGAGTGGATCCGAACATGAAGGTCCACAGCCTGCCCCACGCCACCACACGCTTTCTATAAGCTCCTGGTGGAGCACCGCTCCTGTCGATACCC

1670 1680 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
ACCCCAAGAAAACTTGGAATATAACGTGGAGCCTCAAGAAATCTCACACCCTGACCTGGCAGCCTATTTCTCAGAGTTTACTCGCACTCACTACATCCCAAAGCCAGAGCTCGAGATCCGGTATCCAGAAGATCTGGAG

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940
TTTGTCTATGAAACGGTGCACAATATTTACAGTGGAAAGAAACAGAACATAGATGACTAAAGTCTACAGAGCACATTGCCACCTTTCCTCCTCCTCTATCTTCCAAGACAACGCCACTCCGCAAGAAGACGTCTCCACCG

1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080
CAGGCCTCCGCACCTGCTGCACCACGCAAACCCACTCCACCAGTAGTGCTCGTTCCCTCCTACTAAGTTTAAATACCCTGTCCTCTTCCCCACCTGCAAACACAATCTTCCTCTCCCCCTACACTACTGAATTAATCTGA

2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210 2220
AAGGCACTGTGTCAGTGGCATCCCTTGTATCCTTGTCCTGTGTGACAGTTTGTGACATTCTGTCTTCATCAGGTCTCACAGTCGACGCTCCTGTAATCATTCTTTGTATTCACTCCATTCCCCTGTCTGTCTGCATTT

2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360
GTCTCAGAACATTTCCTTGCCTGGACACATGGGGTTATGCATTTGCAATAATTTCCTTCTGATTTCTCTGTGGAACGTGTTCGGTCCCCAGTGAGCACTGTGCTCTTTTTACCCTCAAGTTAGTTGCATATTCAGAGG

2370 2380 2390 2400 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
TAAAGTTGTGTCTATCTTGGCAGCATCTTACAGATGGACACATTAACAAGCTAATGGTAATTACAATCATTTGAATTTATTTTTTCTAATATGTGAAACACAGATTTCAAGTGTTTTATCTTTTTTTTTTAAATTTA

2510 2520 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
AATGGGAATATAACACAGTTTTCCGTTCCATATTCCTGTCGTTCAGTTTATGCCACATGTGTATAAATCATTAGTTTTCTATTTTATTACATAAAATTGTTTTAGAAAATGCAAATAGTGAACTTTGTGAATGCATTTTTC

2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760 2770 2780
CATACTCATGTACAATTCCTCCATTTTAAATGACTACTTTTATTTTTTAATTTAAAAAATCTACTTCAGTATCATGCAGTAGGTCTTACATCAGTGATGGCTTCTTTTTGTAGTGAGACATACAAATCTGATGTTAATGT

FIG.12C

SUBSTITUTE SHEET (RULE 26)

2790 2800 2810 2820 2830 2840 2850 2860 2870 2880 2890 2900 2910
TTGCTCTTAGAAGTCATACTCCATGCTCTTCAAAGACCAAAAAATGAGGTTTTGCCTTTGTAATCAGGAAAAAAAAAAATTAATGAACCTTAAAAAAAAAAAAAAAGCTTTTCAAGCGAAAAAAGTCGTTTCACACCT

2920 2930 2940 2950 2960 2970 2980 2990 3000 3010 3020 3030 3040 3050
CTTCTTATTCCTTAGAGTCACTTCAAGGCCTGTTTGAATGTGGCAGCTTAGAAAGAGAGAGAATGTCTTTCATTTGAAGAGTGTTGCACTTGTGTGAAAGGAGATGTCCGTGTTGGAATCTGCTTTTCCAACCCGCCAG

3060 3070 3080 3090 3100 3110 3120 3130 3140 3150 3160 3170 3180 3190
GGTCCTGAGCGCAGCAGGACGAACCTGTTGTGCGGTCTTCTGGCAAAGCCTGACCGTGTTGCGACGCCACTGGCTCCTTTCCGAAGTTCTCAGTAACTGAGCCCAGAGTAACTGCAGCCTTTGTGCAGCTCTGGA

3200 3210 3220 3230 3240 3250 3260 3270 3280 3290 3300 3310 3320 3330
GCTCCACCAACTCTCCGCCTGCCAGTTCTCAAGCGAGCTAATCTTGTCATTAATCGATAGAAGCTAACTTCCGAAGTTAGGACCTAGTTACTTTGCTCTCAAACATTTAAAATAATGCAGTTGCTCTAGTGAATGCGCCG

3340 3350 3360 3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470
TTAGGGGCCTGTCTCTGCACCTGTCTGTCCATCTCCATGCAGTATTCTCACCCATGTTGAATGCCTGCTGCTTGTTTACCCTTTGGAAACCCTGGGGTGACCAAGGTTTCGAAAGCCACCTGAGACCACTTCATAGCAA

3480 3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600 3610
CGCAAGCCTTTAAGCAGTTACTAGAAAGACATGCGCATTTGCCCCCTCGCTCCTCCAGCCTGAATGAGCTATTTAACTCCACTGTCCATGTTCCTCATCAGTCCAAATCCAAAGTGATTTCAACCTCCATCTGCAA

3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720 3730 3740 3750
ACGTAACCACTCACAGCACCTGGCCCCCAAGGTTCGGAGGATTGTACACTACTTTCATTTAAAGCGGAAAGTTTGATAATACGCAATTAATTAATATCAATGACATCCATTAATAACAAACCTGACCATGCTGAGAGTT

3760 3770 3780 3790 3800 3810 3820 3830 3840 3850 3860 3870 3880 3890
GCAATTCTTCGTTTCTCGTTTGATTCATTTCCTTTTTTCTTAGACACATCAAACTCAAGAAAGATGGTTTACCTTTACTGACCCAGCTGTACATATCTATCTAGACTGTTTTAAATGTCTTTCTTCATGAATGCTT

3900 3910 3920 3930 3940 3950 3960 3970 3980 3990 4000 4010 4020 4030
CATGCGGCTCCAGCAAGCCTGTATCACCTGTGTAAGTTGCTATTTGGCCACTTTATATTTTTCTAAAAACTGTTTTGGATCCTGTACTCTAATAAATCATAAGTTTCTTTTAAAAATTTTCCAAAACTTTTCTCCAT

4040 4050 4060 4070 4080 4090 4100 4110 4120 4130 4140 4150 4160
TTTAAAAAGCCCTGTTATAAACGTTCAACTTTCACAATGTTAAAATCTTAAATATTTGGATATAGCAACTTCTTTTCTCTTCAATGAATGCCAAGATTTTTTGTACAATGATTAATAAATGCAACTTATCCACAG

FIG.12D

```
        10          20          30          40          50          60
RSTGFRRAGEEWSR*XLAASPGXLRRPAXTFVLSNLAEVVERVLTFLPAKALLRVACVCR

        70          80          90
LWRECVRRVLRTHRSVTWISAGLAEAGHLXGH
```

FIG.13A

```
        10          20          30          40          50          60
CCGTAGTACTGGNTTCCGGCGGGCTGGTGAGGAATGGAGCCGGTAGNTGCTTGCGGCGAG

        70          80          90          100         110         120
TCCCGGGNTCCTCCGTAGACCCGCCGANACCTTCGTGTTGAGTAACCTGGCCGAGGTGGT

        130         140         150         160         170         180
GGAGCGTGTGCTCACCTTCCTGCCCGCCAAGGCGTTCCTGCCGGTGGCCTGCGTGTGCCG

        190         200         210         220         230         240
CTTATGGAGGGAGTGTGTGCGCAGAGTATTGCGGACCCATCGGAGCGTAACCTGGATCTC

        250         260         270
CGCAGGCCTGGCGGAGGCCGGCCACCTGGNGGGGCATT
```

FIG.13B

```
        10         20         30         40         50         60
RPRPVQQQQQQPPQPPPQPPQQQPPQQQPPPPPQQQQQQQPPPPPPPPPLPQERNNVC

        70         80         90        100        110        120
ERDDDVPADMVAEESGPGAQNSPYQLRRKTLLPKRTACPTKNSMEGASTSTTENFGHRAK

       130        140        150        160        170        180

RARVSGKSQDLSAAPAEQYLQELKLPDEVVLKIFSYLLEQDLCRAACVCKRFSELANDPNL

       190
WKRLYMEVFEYTRPMMH
```

FIG.14A

```
        10         20         30         40         50         60

GCGGCCGCGCCCCGGTGCAGCAACAGCAGCAGCCCCCGCAGCAGCCGCCGCCGCAGCC

        70         80         90        100        110        120
GCCCCAGCAGCAGCCGCCCCAGCAGCAGCCTCCCCCGCCGCCGCAGCAGCAGCAGCA

       130        140        150        160        170        180
GCAGCCTCCGCCGCCGCCACCGCCGCCTCCGCCGCTGCCTCAGGAGCGGAACAACGTCGG

       190        200        210        220        230        240
CGAGCGGGATGATGATGTGCCTGCAGATATGGTTGCAGAAGAATCAGGTCCTGGTGCACA

       250        260        270        280        290        300
AAATAGTCCATACCAACTTCGTAGAAAAACTCTTTTGCCGAAAAGAACACGCGTGTCCCAC

       310        320        330        340        350        360
AAAGAACAGTATGGAGGCGCCTCAACTTCAACTACAGAAAACTTTGGTCATCGTGCAAA

       370        380        390        400        410        420
ACGTGCAAGAGTGTCTGGAAAATCACAAGATCTATCAGCAGCACCTGCTGAACAGTATCT

       430        440        450        460        470        480
TCAGGAGAAACTGCCAGATGAAGTGGTTCTAAAAATCTTCTCTTACTTGCTGGAACAGGA

       490        500        510        520        530        540
TCTTTGTAGAGCAGCTTGTGTATGTAAACGCTTCAGTGAACTTGCTAATGATCCCAATTT

       550        560        570        580        590
GTGGAAACGATTATATATGGAAGTATTTGAATATACTCGCCCTATGATGCAT
```

FIG.14B

```
      10        20        30        40        50        60
RPRPGLRGGRAPCEVTMEAGGLPLELWRMILAYLHLPDLGRCSLVCRAWYELILSLDSTR

      70        80        90       100       110       120
WRQLCLGCTECRHPNWPNQPDVEPESWREAFKQHYLASKTWTKNALDLESSICFSLFRRR

     130       140       150       160       170
RERRTLSVGPGREFDSLGSALAWASLYDRIVLFPGVYEEQGEIILKVPVEIVGQGKLG
```

FIG.15A

```
      10        20        30        40        50        60
GCGGCCGCCGCCCCGGACTCCGCGGTGGGCGAGCGCCCTGTGAGGTGACCATGGAGGCTGG

      70        80        90       100       110       120
TGGCCTCCCCTTGGAGCTGTGGCGCATGATCTTAGCCTACTTGCACCTTCCCGACCTGGG

     130       140       150       160       170       180
CCGCTGCAGCCTGGTATGCAGGGCCTGGTATGAACTGATCCTCAGTCTCGACAGCACCCG

     190       200       210       220       230       240
CTGGCGGCAGCTGTGTCTGGGTTGCACCGAGTGCCGCCATCCCAATTGGCCCAACCAGCC

     250       260       270       280       290       300
AGATGTGGAGCCTGAGTCTTGGAGAGAAGCCTTCAAGCAGCATTACCTTGCATCCAAGAC

     310       320       330       340       350       360
ATGGACCAAGAATGCCTTGGACTTGGAGTCTTCCATCTGCTTTTCTCTATTCCGCCGGAG

     370       380       390       400       410       420
GAGGGAACGACGTACCCTGAGTGTTGGGCCAGGCCGTGAGTTTGACAGCCTGGGCAGTGC

     430       440       450       460       470       480
CTTGGCCATGGCCAGCCTGTATGACCGAATTGTGCTCTTCCCAGGTGTCTACGAAGAGCA

     490       500       510       520       530
AGGTGAAATCATCTTGAAGGTGCCTGTGGAGATTGTAGGGCAGGGGAAGTTGGGTGA
```

FIG.15B

```
        10         20         30         40         50         60
ETETAPLTLESLPTDPLLLILSFLDYRDLINCCYVSRRLSQLSSHDPLWRRHCKKYWLIS

        70         80         90        100        110        120
EEEKTQKNQCWKSLFIDTYSDVGRYIDHYAAIKKASGMISRNIWSPGVLGWVLSLKEGCS

       130        140        150        160        170        180
RGRPRCQGSADWAASFLDDYRCSYRIHNGQKLVGSWGYWEAWHCLITIVLKIC*TSIQLP

       190        200        210        220        230        240
EIPAETGTEILSPFNFCIHTGLSQYIAVEAAEG*NKNEVFYQCQTVERVFKYGIKMCSDG

       250
CINGMH*VFS
```

FIG.16A

```
        10         20         30         40         50         60
GAGACCGAGACGGCGCCGCTGACCCTAGAGTCGCTGCCCACCGATCCCCTGCTCCTCATC

        70         80         90        100        110        120
TTATCCTTTTTGGACTATCGGGATCTAATCAACTGTTGTTATGTCAGTCGAAGATTAAGC

       130        140        150        160        170        180
CAGCTATCAAGTCATGATCCGCTGTGGAGAAGACATTGCAAAAAATACTGGCTGATATCT

       190        200        210        220        230        240
GAGGAAGAGAAAACACAGAAGAATCAGTGTTGGAAATCTCTCTTCATAGATACTTACTCT

       250        260        270        280        290        300
GATGTAGGAAGATACATTGACCATTATGCTGCTATTAAAAAGGCCTCGGGAATGATCTCA

       310        320        330        340        350        360
AGAAATATTTGGAGCCCAGGTGTCCTCGGATGGGTTTTATCTCTGAAAGAGGGGTGCTCG

       370        380        390        400        410        420
AGAGGAAGACCTCGATGCTGTGGAAGCGCAGATTGGGCTGCAAGTTTCCTGGACGATTAT

       430        440        450        460        470        480
CGATGTTCATACCGAATTCACAATGGACAGAAGTTAGTTGGTTCCTGGGGTTATTGGGAA

       490        500        510        520        530        540
GCATGGCACTGTCTAATCACTATCGTTCTGAAGATTTGTTAGACGTCGATACAGCTGCCG

       550        560        570        580        590        600
GAGATTCCACCAGAGACACGGACTGAAATACTGTCTCCCTTTAACTTTTGCATACATACT

       610        620        630        640        650        660
GGTTTGAGTCAGTACATAGCAGTGGAAGCTGCAGAGGGTTGAAACAAAAATGAAGTTTTC

       670        680        690        700        710        720
TACCAATGTCAGACAGTAGAACGTGTGTTTAAATATGGCATTAAGATGTGTTCTGATGGT

       730        740        750
TGTATAAATGGCATGCATTAGGTATTTTCAG
```

FIG.16B

```
        10         20         30         40         50         60
GSGFRAGGWPLTMPGKHQHFQEPEVGCCGKYFLFGFNIVFWVLGALFLAIGLWAWGEKGV

        70         80         90        100        110        120
LSNISALTDLGGLDPVWLVCGSWRRHVGAGLCWAAIGALRENTFLLKFFXXFLGLIFFLE

LA
```

FIG.17A

```
        10         20         30         40         50         60
GGCTCCGGTTTCCGGGCCGGCGGGTGGCCGCTCACCATGCCCGGNAACCACCAGCATTTC

        70         80         90        100        110        120
CAGGAACCTGAGGTCGGCTGCTGCGGCAAATACTTCCTGTTTGGCTTCAACATTGTCTTC

       130        140        150        160        170        180
TGGGTGCTGGGAGCCCTGTTCCTGGCTATCGGCCTCTGGGCCTGGGGTGAGAAGGGCGTT

       190        200        210        220        230        240
CTCTCGAACATCTCAGCGCTGACAGATCTGGGAGGCCTTGACCCCGTGTGGCTTGTTTGT

       250        260        270        280        290        300
GGTAGTTGGAGGCGTCATGTCGGTGCTGGGCTTTGCTGGGCTGCAATTGGGGCCCTCCGG

       310        320        330        340        350        360
GAGAACACCTTCCTGCTCAAGTTTTTCTNCGNGTTCCTCGGTCTCATCTTCTTCCTGGAG

CTGGCAAC
```

FIG.17B

```
        10         20         30         40         50         60
AAAAAAYLDELPEPLLLRVLAALPAAELVQACRLVCLRWKELVDGAPLWLLKCQQEGLVP

        70         80         90        100        110        120
EGGVEEERDHWQQFYFLSKRRRNLLRNPCGEEDLEGWCDVEHGGDGWRVEELPGDSGVEF

       130        140        150        160        170        180
THDESVKKYFASSFEWCRKAQVIDLQAEGYWEELLDTTQPAIVVKDWYSGRSDAGCLYEL

       190        200        210        220        230        240
TVKLLSEHENVLAEFSSGQVAVPQDSDGGGWMEISHTFTDYGPGVRFVRFEHGGQSVYW

       250
KGWFGARVTNSSVWVEP*
```

FIG.18A

```
          10         20         30         40         50         60
GCGGCGGCCGGCCGCCGCGTACCTGGACGAGCTGCCCGAGCCGCTGCTGCTGCGCGTGCTGGCCGCACTG
70         80         90        100        110        120        130
CCGGCCGCCGAGCTGGTGCAGGCCTGCCGCCTGGTGTGCCTGCGCTGGAAGGAGCTGGTGGACGGCGCC
140        150        160        170        180        190        200
CCGCTGTGGCCTGCTCAAGTGCCAGCAGGAGGGGCTGGTGCCCGAGGGCGGCGTGGAGGAGGAGCGCGAC
210        220        230        240        250        260        270
CACTGGCAGCAGTTCTACTTCCTGAGCCAAGCGGCGCCCGCAACCTTCTGCGTAACCCGTGTGCGGAAGAG
280        290        300        310        320        330        340
GACTTGGAAGGCTGCTGTACGTGGAGCATGCTGCCGCACGGCTGGAGGGTGGAGGAGCTGCCTGCAGAC
350        360        370        380        390        400        410
AGTGCGGGTGGAGTTCACCCACGATGACAGCGTCAAGAAGTACTTCGCCCTCCTCCTTTGAGTGGTCTCGC
420        430        440        450        460        470        480
AAAGCACAGGTCATTGACCTGCAGGCTGAGGGCTACTGCGGAGGAGCTGCTGGACACGCACTCACGCCGCC
490        500        510        520        530        540        550
ATCGTGGTGAAGGACTGGTACTCGGGCCGCAGCGACGCTGGTTGCCTCTACGACCTCACCGTTAAGCTA
560        570        580        590        600        610        620
CTGTCCGACCACGACGAACGTGCTGGCTGAGTTCAGCAGCGGGCAGGTGGCAGTGCCCCAAGACAGTGAC
630        640        650        660        670        680        690
GGCGGGGGCTGGATGGAGATCTCCCACACCTTCACCGACTACGGGCCGGGCGTCCGCTTCGTCCGCTTC
          700        710        720        730        740        750
GAGCACGGGGGGCAGGGCTCCGTCTACTGGAAGGCCTGGTTCGGGGCCCGGGTGACCAACAGCAGCGTG
760        770
TGGGTAGAACCCTGA
```

FIG.18B

```
        10         20         30         40         50         60
MGEKAVPLLRRRRVKRSCPSCGSELGVEEKRGKGNPISIQLFPPELVEHIISFLPVRDLV

        70         80         90        100        110        120
ALGQTCRYFHEVCDGEGVWRRICRRLSPRLQDQDTKGLYFQAFGGRRRCLSKSVAPLLAH

       130        140        150        160        170        180
GYRRFLPTKDHVFILDYVGTLFFLKNALVSTLGQMQWKRACRYVVLCRGAKDFASDPRCD

       190        200        210        220        230        240
TVYRKYLYVLATREPQEVVGTTSSRACDCVEVYLQSSGQRVFKWTFHHSMTFKQIVLVGQ

       250        260        270        280        290        300
ETQRALLLLTEEGKIYSLVVNETQLDQPRSYTVQLALRKVSHYLPHLRVACMTSNQSSTL

       310
YVTDPILCSWLQPPWPGG
```

FIG.19A

```
          10         20         30         40         50         60
ATGGGCGAGAAGGCGGTCCCTTTGCTAAGGAGGAGCGCGGTGAAGAGAAGCTGCCCTTCTTGTGGCTCG

70         80         90        100        110        120        130
GAGCTTGGGGTTGAAGAGAAGAGGGGGAAAGGAAATCCGATTTCCATCCAGTTGTTCCCCCCAGAGCTG

140        150        160        170        180        190        200
GTGGAGCATATCATCTCATTCCTCCCAGTCAGAGACCTTGTTGCCCTCGGCCAGACCTGCCGCTACTTC

210        220        230        240        250        260        270
CACGAAGTGTGCGATGGGGAAGCCGTGTGGAGACGCATCTGTCGCAGACTCAGTCCGCGCCTCCAAGAT

280        290        300        310        320        330        340
CAGGACACGAAGGGCCTGTATTTCCAGGCATTTGGAGGCCGCCGCCGATGTCTCAGCAAGAGCGTGGCC

350        360        370        380        390        400        410
CCCTTGCTAGCCCACGGCTACCGCCCGCTTCTTGCCCACCAAGGATCACGTCTTCATTCTTGACTACGTG

420        430        440        450        460        470        480
GGGACCCTCTTCTTCCTCAAAAATGCCCTGGTCTCCACCCTCGGCCAGATGCAGTGGAAGCGGGCCTGT

490        500        510        520        530        540        550
CGCTATGTTGTGTTGTGTCGTGGAGCCAAGGATTTTGCCTCGGACCCAAGGTGTGACACAGTTTACCGT

560        570        580        590        600        610        620
AAATACCTCTACGTCTTGGCCACTCGGGAGCCGCAGGAAGTGGTGGGTACCACCAGCAGCCGGGCCTGT

630        640        650        660        670        680        690
GACTGTGTTGAGGTCTATCTGCAGTCTAGTGGGCAGCGGGTCTTCAAGATGACATTCCACCACTCAATG

700        710        720        730        740        750
ACCTTCAACAGATCGTGCTGGTTGGTCAGGAGACCCAGCGGGCTCTACTGCTCCTCACAGAGGAAGCA

760        770        780        790        800        810        820
AAGATCTACTCTTTGGTAGTGAATGAGACCCAGCTTCACCAGCCACGCTCCTACACGGTTCAGCTGGCC

830        840        850        860        870        880        890
CTGAGGAAGGTGTCCCACTACCTGCCTCACCTGCGCGTGGCCTGCATGACTTCCAACCAGAGCAGCACC

900        910        920        930        940        950
CTCTACGTCACAGATCCTATTCTGTGCTCTTGGCTACAACCACCTTGGCCTGGTGGATGA
```

FIG.19B

```
        10         20         30         40         50         60
RGGSEGRGRGREKRARGARRKRKQGGREARAADGEGGSGPGAEAGARTRPREEAEGGGSV

        70         80         90        100        110        120
EEGARGIIKGDEGSVGAGKEAQGRKYGKEEMRVRARRREGARPGRVQGQGGQVWAYIPGT

       130        140        150        160        170        180
GAAWAAAARFEEEEAARESAACPAAGPALWRLPEVLLLHMCSYLDMRALGRLAQVYRWLW

       190        200        210        220        230        240
HFTNCDLLRRQIAWASLNSGFTRLGTNLMTSVPVKVSQNWIVGCCREGILLKWRCSQMPW

       250        260        270        280        290        300
MQLEDDALYISQANFILAYQFRPDGASLNRQPLGVSAGHDEDVCHFVLATSHIVSAGGDG

       310        320        330        340        350        360
KIGLGKIHSTFAAKYWAHEQEVNCVDCKGGIISFGSRDRTAKVWPLASGQLGQCLYTIQT

       370        380        390        400        410        420
EDQIWSVAIRPLLSSFVTGTACCGHFSPLKIWDLNSGQLMTHLDRDFPPRAGVLDVIYES

       430        440        450        460        470        480
PFALLSCGYDTYVRYWDCRTSVRKCVMEWEEPHNSTLYCLQTDGNHLLATGSSFYSVVRL

       490        500        510        520        530
WDRHQRACPHTFPLTSTRLGSPVYCLHLTTKHLYAALSYNLHVLDIQNP*
```

FIG.20A

SUBSTITUTE SHEET (RULE 26)

```
          10         20         30         40         50         60         70         80         90
CGAGGGGCGAAGCGAAGCGAAGGGCGAAGAGGAAGGGAAAAGCGACGGAGAGGGGCAAGGCGGAAGAGAGGAAGCAGGGCGGAAGGGAAGCCCGGCCG

     100        110        120        130        140        150        160        170        180
CAGACGGCGAAGGAGCCAGCGGCCGGGGGCTGAGCCGGGAGCGAGGACACGCCCAAGAGAGGAAGCAGACGGAGGCGGAAGCGTGGAGGAAGG

190        200        210        220        230        240        250        260        270        280
GGCGAGAGGCATCATCAAAGGAGATGACGGGAGCGTAGGGGCCGGAAAGACGCACAACGAAGAAAGTATGCGAAGGAGGAATGGAGGTCAGG

     290        300        310        320        330        340        350        360        370
GCTAGGCGGCGGGAGGGCGCCAGGCCGGGAAGAGTACAAGGACAAGGAGGTCAGTTTGGCCTACATCCCGGGGACAGGGGCGGCCATGGCGG

 380        390        400        410        420        430        440        450        460        470
CGGCAGCCAGGGAGGAGGAGGAGGAGCGGCTCGGGAGTCAGCCGCCTGCCCGGCTGCCGGGCCAGCGCTCTGGCGCCTGCCGGAAGTGCTGCT

          480        490        500        510        520        530        540        550        560
GCTGCACATGTGCTCCTACCTCGACATGCGGGCCCTCGGCCGCCCTGCCCCAGGTGTACCGCTGGCTGTGGCACTTCACCAACTGCGACCTGCTC

     570        580        590        600        610        620        630        640        650
CGGCGCCAGATAGCCTGGGCCTCGCTCAACTCCGGCTTCACGCGGCTCGGCACCAACCTGATGACCAGTGTCCCAGTGAAGGTGTCTCAGAACT

660        670        680        690        700        710        720        730        740        750
GGATAGTCGCGCTGCTGCCGAGAGCGGATTCTGCTGAAGTGCAGATGCAGTCAGATCCCCTGGATGCAGCTAGAGGATGATGCTTTGTACATATC

     760        770        780        790        800        810        820        830        840
CCAGCCTAATTTCATCCTGGCCTACCAGTTCCGTCCAGATGGTGCCAGCTTGAACCGTCAGCCTCTGCGAGTCTCTGCTGGCATGATGAGGAC

 850        860        870        880        890        900        910        920        930        940
GTTTGCCACTTTGTGCTGGCCACCTCGCATATTGTCAGTGCAGGAGGAGATGGGAAGATTGGCCTTGGTAAGATTCACAGCACCTTCGCTGCCA
```

FIG.20B

950 960 970 980 990 1000 1010 1020 1030
AGTACTGGCCTCATGCAACAGGAGGTGAACTGTGTGGATTGCAAAGGGGCATCATCATTGGCTCCAGGACAGCACGGCCAAGGTGTGCCC

1040 1050 1060 1070 1080 1090 1100 1110 1120
TTTGGCCTCAGGCCAGCTGGGGCAGTGTTTATACACCATCCAGACTGAAGACCAAATCTGGTCTGTTGCTATCAGGCCATTACTCAGCTCTTTT

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
GTGACAGGCAACGGCTTGTTGTGGGCACTTCTCACCCCTGAAAATCTGGCACCTCAACAGTGGCCAGCTGATGACACACTTGGACAGAGACTTTC

1230 1240 1250 1260 1270 1280 1290 1300 1310
CCCCAAGGGCTGGGGTGCTGGAATGTCATATATGACTCCCCTTTCGGCACTGCTCCTGTGGCTATGACACCTATGTTCGCTACTGGGACTGCCG

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
CACCAGTGTCCGGAAATGTGTCATGGAGTGGGAGGAGGCCCCACAACAGCACCCTGTACTGCCTGCAGACAGATGGCAACCACTTGCCTGCCACA

1420 1430 1440 1450 1460 1470 1480 1490 1500
GGTTCCTCCTTCTATAGCGTTGTAGGCTGTGGGACCGGCACCAAAAGGGCCTGCCCCCACACCTTCCCGCTGACGTCGACCGGCCTCGGCAGCC

1510 1520 1530 1540 1550 1560 1570 1580 1590
CTGTGTACTGCCTGCATCTCACCACCAAGCATCTCTATGCTGCGCTGTCTTACAACCTCCACGTCCTGGATATTCAAAACCCGTGA

FIG.20C

```
        10         20         30         40         50         60
LILTSVLLFGRHGYCTLGEAFNRLDFSSAIQDIRTFNYVVKLLQLIAKSQLTSLSGVAQK

        70         80         90        100        110        120
NYFNILDKIVQKVLDDHHNPRLIKDLLQDLSSTLCILIRGVGKSVLVGNINIWICRLETI

       130        140        150        160        170        180
LAWQQQLQDLQMTKQVNNGLTLSDLPLHMLNNILYRFSDGWDIITLGQVTPTLYMLSEDR

       190        200        210        220        230        240
QLWKKLCQYHFAEKQFCRHLILSEKGHIEWKLMYFALQKHYPAKEQYGDTLHFCRHCSIL

       250        260        270
FWKDSGHPCTAADPDSCFTPVSPQHFIDLFKF
```

FIG.21A

```
         10         20         30         40         50         60
GCATTGCTATAATTTTACTATACTCTCATCTAAATCTAAAATCAGTCTTCAAAATAAAAACAAATTGTC

70         80         90        100        110        120        130
CTTTGCCAAAAATTTTTTTAATGGCACAATTAATTGACATTAACTGCCAATTCTTTTTGGCTAATTGAC

140        150        160        170        180        190        200
TAATTTTAACTTCTGTGTTGCTTTTCCAGAGGCATGGCTATTGCACCTTGGGAGAAGCCTTTAATCGGT

210        220        230        240        250        260        270
TAGACTTCTCAAGTGCAATTCAAGATATCCGAACGTTCAATTATGTGGTCAAACTGTTGCAGCTAATTG

280        290        300        310        320        330        340
CAAAATCCCAGTTAACTTCATTGAGTGGCGTGGCACAGAAGAATTACTTCAACATTTTGGATAAAATCG

350        360        370        380        390        400        410
TTCAAAAGGTTCTTGATGACCACCACAATCCTCGCTTAATCAAAGATCTTCTGCAAGACCTAAGCTCTA

420        430        440        450        460        470        480
CCCTCTGCATTCTTATTAGAGGAGTAGGGAAGTCTGTATTAGTGGCAAACATCAATATTTGGATTTGCC

490        500        510        520        530        540        550
GATTAGAAACTATTCTCGCCTGGCAACAACAGCTACAGGATCTTCAGATGACTAAGCAAGTGAACAATG

560        570        580        590        600        610        620
GCCTCACCCTCAGTGACCTTCCTCTGCACATGCTGAACAACATCCTATACCGGTTCTCAGACGGATGGG

630        640        650        660        670        680        690
ACATCATCACCTTAGGCCAGGTGACCCCCACGTTGTATATGCTTAGTGAAGACAGACAGCTGTGGAAGA

700        710        720        730        740        750
AGCTTTGTCAGTACCATTTTGCTGAAAAGCAGTTTTGTAGACATTTGATCCTTTCAGAAAAAGGTCATA

760        770        780        790        800        810        820
TTGAATGGAAGTTGATGTACTTTGCACTTCAGAAACATTACCCAGCGAAGGAGCAGTACGGAGACACAC

830        840        850        860        870        880        890
TGCATTTCTGTCGGCACTGCAGCATTCTCTTTTGGAAGGACTCAGGACACCCCTGCACGGCGGCCGACC

900        910        920        930        940        950        960
CTGACAGCTGCTTCACGCCTGTGTCTCCGCAGCACTTCATCGACCTCTTCAAGTTTAAGGGCTGCCCC
```

FIG.21B

970 980 990 1000 1010 1020 1030
TGCCATCCCTATTGGAGATTGTGAATCCTGCTGTCTGTGCAGGGCTCATAGTGAGTGTTCTGTGAGGTG

1040 1050 1060 1070 1080 1090 1100
GGTGGAGACTCCTCGGAAGCCCCTGCTTCCAGAAAGCCTGCGAAGAACTCCCCTTCTGCAAAGGGGGGA

1110 1120 1130 1140 1150 1160 1170
CTGCATGGTTGCATTTTCATCACTGAAACTCAGAGGCCAAGGAAATCATTTCTACTTCTTTAAAAACTC

1180 1190 1200 1210
CTTCTAAGCATATTAAAATGTGAAATTTTGCGTACTCTCTC

FIG.21C

```
        10         20         30         40         50         60
YGSEGKGSSSISSDVSSSTDHTPTKAQKNVATSEDSDLSMRTLSTPSPALICPPNLPGFQ

        70         80         90        100        110        120
NGRGSSTSSSSITGETVAMVHSPPPTRLTHPLIRLASRPQKEQASIORLPDHSMVQIFSF

       130        140        150        160        170        180
LPTNQLCRCARVCRRWYNLAWDPRLWRTIRLTGETINVDRALKVLTRRLCQDTPNVCLML

       190        200        210        220        230        240
ETVTVSGCRRLTDRGLYTIAQCCPELRRLEVSGCYNISNEAVFDVVSLCPNLEHLOVSGC

       250        260        270        280        290        300
SKVTCISLTREASIKLSPLHGKQISIRYLOMTDCFVLEDEGLHTIAAHCTQLTHLYLRRC

       310        320        330        340        350        360
VRLTDEGLRYLVIYCASIKELSVSOCRFVSDFGLREIAKLESRLRYLSIAHCGRVTDVGI

       370        380        390        400        410        420
RYVAKYCSKLRYLNARGCEGITDHGVEYLAKNCTKLKSLDIGKCPLVSDTGLECLALNCF

       430        440        450        460        470        480
NLKRLSLKSCESITGQGLQIVAANCFOLQTLNVQDCEVSVEALRFVKRHCKRCVIEHTNP

AFF
```

FIG.22A

SUBSTITUTE SHEET (RULE 26)

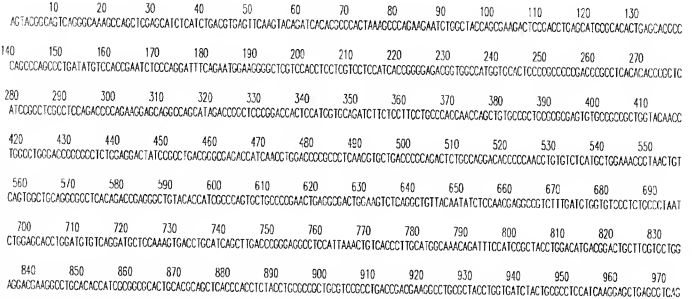

FIG.22B

1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520
GCGCTTTGTCAAACGCCACTGCAAGCGCTGCGTCATCGAGCACACCAACCCGGCTTTCTTCTGAAGGACAGAGTTCATCCGGCGTTGTATTCACACAAACCTGAACAAAGCAAATTTTTTTAAAAGCACCGTATGTAA

1530 1540 1550 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660
GCACCGACACCCACTCAAAACAGCTCTTTCTTCCGGGAAGGTTATTAGGAATCTGGCCTTTATTTTTCCTCATTTCTCATGGGCAACAGAGGCCAAAGAAACGAAGCAAGACAAACAGCAAACAGGCATTTTGGTCAGG

1670 1680 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
TCATTTGTACGCAGTTTCTCTTCTCACAAAAGATGTACTTAAGCAGGCTGATCGCTGTTCCTTGAGCAAGGCGCTTACTCTCCTCCGCTCAGGCCCCCAAGGCCGCCCTTTCCCTCGCACACAGGCCCCACCCCCACAG

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940
TTCCACGCCCCCCCCCCAAGGCCACACCCTCCCTCCCTAGAGCAGCAGCGAGGATCCATCATCAGAATCACAGTGCTCTCCAGACCTCCTCTCTAAACTGCTTCATTGACCTAAGTCACTCTCTTCAATCCCACACCCA

1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080
TGGACATTCTTGTCAACTCAATACCATAGCACTTTGCATAGGCAAAATACTTTTCAGGCCTTTTTAAAAAATTCATTACAGCAAACAGCTGGGCAAGGACATGCAGTCCTCCCCCAGCTCTGTCAATGACTATGACCTT

2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210 2220
GGCCAAAGCACTTCACTGCTCTGGGCTGCAGCTTCCAGCACTGAATCAGAGGCCACACAGCCCAAAGATTAGCTTCATGTCCATTATAGCATTGAGGGAGCAGAGATACCCATACACAGAAGCACCTTGGCATAGAGCA

2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360
CCCAGGCATCGACCTCTTCCAGGAGAACTGATTCTGTGGATGGATGTGATTTCAGGAGATTGTGCAGTGCCAGCATCAGTCCATAAAGGGTCCTGTATGTCCTTTGGCTGCAAATCACCCACTTCCCTGTGTTTCAGTG

2370 2380 2390 2400 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
GGAGAATTTCCTCTCCCACCTCCTCACATCCTCTTTTGCCAGGCTGGATGCTGTCGTCTCTGTACACAAATACTTTCTGCATTCCCCCCTCCACACCATCCTAGCGAGGCACCAGCACACCTAATCACAGCAAAGCCCA

2510 2520 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
GATCCCCCCATCAGTTGCTTTTACTCAGTGTTTTCAAATACGAGTAAAGGCCCTTGCAATTTTTAATTAACAAGCAAGGCCCAAGGGAACACATGTCCTCAAAAGTTTTTCTGATCCCTCGCCTTCCACACCTGGCATC

2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760 2770 2780
CATCAGGCACATCTGTCCTACAGCTGGCAGAGACAGATGCCTCGGTTCTTTGTCATTCAGATTGCATTTGACCTCTTCTCATCTATTTATTTCTTTATACATCCAGACTTCATCACATGAAGCCTATTGGGGTTAAGTT

FIG.22C

SUBSTITUTE SHEET (RULE 26)

```
      2790       2800       2810       2820       2830       2840       2850       2860       2870       2880       2890       2900       2910
TGTAAGTGTTTAATTGTGCAAATTGCCACCCTGTGTACCTCCTCCATGTCTGTCTGCGTGTTTTCCACCAAAGAATCCAAAGCAGACTTCCAGTGTTTAAATTCTCTTCACTCAACAATCCCAGATGAATGCAACAGCG

2920       2930       2940       2950       2960       2970       2980       2990       3000       3010       3020       3030       3040       3050
GAACACACTGACATGACTTAGACTCTGCTCCACCAACCAGACCCTTGGAAAGGAATACTAAAATCATTACAAGGTATGGATTTTAAATGCATGAAACTTCAAATTATCTTATTTGGATAGAAGTCTATATTCTACCCTC

3060       3070       3080       3090       3100       3110       3120       3130       3140       3150       3160       3170       3180       3190
ATTTGCATGAAGTCAGATAGCCAGAAGAAATTCCATTCCTGGTTTTCAGCAAATTCACTTGTCTTTTGCTAATAAACACATGGCCCTTTCCCAGATTATTCTCTAGCCAAGCCCCACCTTTGTTACGTTGAAATCCCTC

3200       3210       3220       3230       3240       3250       3260       3270       3280       3290       3300       3310       3320       3330
ATTTATTTCTTCTCAAAATGCCCATTATCCAAATGCAGAACCTCTGCATCTCCAAGCCAGTTATGCTCAATTTGTCAAACTTACACACCCTTGACAACTCCACTCCTACTGTAGGCTCCTGTCCATACTGTCGTCTTC

3340       3350       3360       3370       3380       3390       3400       3410       3420       3430       3440       3450       3460       3470
TGTGGGGGATGCGACAGCTTAGTGTCATCAGCTGGTGTCTGCCCAGCAGCTTTCTTTCAAACATCATGGCCTCCCATCCAATCAACATCATCAAATTACATGTGTAATCAAGCCTCTCTGCCATCGCCGAAATGAATCAT

3480       3490       3500       3510       3520       3530       3540       3550       3560       3570       3580       3590       3600       3610
TTAGCTAGCCCAGCATCTAGTCAAAGCCACAGATTTAAAACCATGAAAGAAGTTGAAGGCAGCATTCCTCAGCTCTGTCACTTGTGACCCTATTTGAAGTTTCAGGATTTGCGTGTCACAAAGCATTGTCCCTAATCC

3620       3630       3640       3650       3660       3670       3680       3690       3700       3710       3720       3730       3740       3750
TTGGCCTGGGTCTTCCGAGTGACCTGGTTTAATACTCTGACAATGAGCACGGAGATCCAGAGAATGAATCCCTGACCCATACCTAAACTGTCTTCCAAACATGAGACAAAGCTGACTGTTCACACTGATTGCCCA

3760       3770       3780       3790       3800       3810       3820       3830       3840       3850       3860       3870       3880       3890
CCACATACCGTCTTGCCAGTTTCTTCTTTTCTCCCAGTCTCCTGTTCATCCATTCTGTTCTCCCTTGGGGTGGCAATCTATGATGCAGCTTACTCCGGAAACACCTCAGCAGATTTTTCGCAGACCAAACCAAAGTCTC

3900       3910       3920       3930       3940       3950       3960       3970       3980       3990       4000       4010       4020       4030
ACTAGGAAATTTATCTGTTTTAAAACATTGCTTCCTTCCTGGCTCTGCTAAATTGAATGCTCATTGTTTGTTGTTGTTTTTAATTCTAATGTTCAAATCACTGCGTCCTGTATGAATCTAGAAAGCCTTAATTTA

4040       4050
CTACCAAGAAATAAAGCAATATGTTCGT
```

FIG.22D

```
        10         20         30         40         50         60
AAAPAPAPAPTPTPEEGPDAGWGDRIPLEILVQIFGLLVAADGPMPFLGRAARVCRRWQE

        70         80         90        100        110        120
AASQPALWHTVTLSSPLVGRPAKGGVKAEKKLLASLEWLMPNRFSQLQRLTLIHWKSQVH

       130        140        150        160        170        180
PVLKLVGECCPRLTFLKLSGCHGVTADALVMLAKACCQLHSLDLQHSMVESTAVVSFLEE

       190        200        210        220        230        240
AGSRMRKLWLTYSSQTTAILGALLGSCCPQLQVLEVSTGINRNSIPLQLPVEALQKGCPQ

       250        260        270        280
LQVLRLLNLMWLPKPPGRGVAPGPGFPSLEELCLASSTCNFVS
```

FIG.23A

```
        10         20         30         40         50         60
TGCGGCCGCGCCCGCACCCGCACCGGCACCCACGCCCACGCCCGAGGAAGGGCCCGACGCGGGCTGGGG

70         80         90         100        110        120        130
AGACCGCATTCCCTTGGAAATCCTGGTGCAGATTTTCGGGTTGTTGGTGGCGGCCGGACGGCCCCCATGCC

140        150        160        170        180        190        200
CTTCCTGGGCAGGGCTGCGCGCGGTGTGCCGCCGCTGGCAGCAGGCCGCTTCCCAACCCGCGCTCTGGCA

210        220        230        240        250        260        270
CACCGTGACCCTGTCGTCCCGCTGGTCGGCCGGCCTGCCAAGGCGCGGGTCAAGGCGGAGAAGAAGCT

280        290        300        310        320        330        340
CCTTGCTTCCCTGGAGTGGCTTATGCCCAATCGGTTTTCACAGCTCCAGAGGCTGACCCTCATCCACTG

350        360        370        380        390        400        410
GAAGTCTCAGGTACACCCCGTGTTGAAGCTGGTAGGTGAGTGCTGTCCTCGGCTCACTTTCCTCAAGCT

420        430        440        450        460        470        480
CTCCGGCTGCCACGGTGTGACTGCTGACGCTCTGGTCATGCTAGCCAAAGCCTGCTGCCAGCTCCATAG

490        500        510        520        530        540        550
CCTGGACCTACAGCACTCCATGGTGGAGTCCACAGCTGTGGTGAGCTTCTTGGAGGAGGCAGGGTCCCG

560        570        580        590        600        610        620
AATGCGCAAGTTGTGGCTGACCTACAGCTCCCAGACGACAGCCATCCTGGGGCGCATTGCTGGGCAGCTG

630        640        650        660        670        680        690
CTGCCCCCAGCTCCAGGTCCTGGAGGTGAGCACCGGCATCAACCGTAATAGCATTCCCCTTCAGCTGCC

700        710        720        730        740        750
TGTCGAGGCTCTGCAGAAAGGCTGCCCTCAGCTCCAGGTGCTGCGGCTGTTGAACCTGATGTGGCTGCC

760        770        780        790        800        810        820
CAAGCCTCCGGGACGACGGGTGGCTCCCGGACCAGGCTTCCCTAGCCTAGAGGAGCTCTGCCTGGCGAG

830        840        850
CTCAACCTGCAACTTTGTGAGC
```

FIG.23B

```
        10         20         30         40         50         60
QHCSQKDTAELLRGLSLWNHAEERQKFFKYSVDEKSDKEAEVSEHSTGITHLPPEVMLSI

        70         80         90        100        110        120
FSYLNPQELCRCSQVSMKWSQLTKTGSLWKHLYPVHWARGDWYSGPATELDTEPDDEWVK

       130        140        150        160        170        180
NRKDESRAFHEWDEDADIDESEESAEESIAISIAQMEKRLLHGLIHNVLPYVGTSVKTLV

       190        200        210        220        230        240
LAYSSAVSSKMVRQILELCPNLEHLDLTQTDISDSAFDSWSWLGCCQSLRHLDLSGCEKI

       250        260        270        280        290        300
TDVALEKISRALGILTSHQSGFLKTSTSKITSTAWKNKDITMQSTKQYACLHDLTNKGIC

       310        320        330        340        350        360
EEIDNEHPWTKPVSSENFTSPYVWMLDAEDLADIEDTVEWRHRNVESLCVMETASNFSCS

       370        380        390        400        410        420
TSGCFSKDIVGLRTSVCWQQHCASPAFAYCGHSFCCTGTALRTMSSLPESSAMCRKAART

       430        440        450        460        470        480
RLPRGKDLIYFGSEKSDQCTGRVLLFLSLSGCYQITDHGLRVLTLGGGLPYLEHLNLSGC

       490        500        510        520        530        540
LTITGAGLQDLVSACPSLNDEYFYYCDNINGPHADTASGCQNLQCGFRACCRSGE*PLTS

       550        560        570        580        590
DLCLLHLAEQAFFHALYS*HISCVNHPFLSVTCFGPIXYNFRNLNYQXIVML
```

FIG.24A

SUBSTITUTE SHEET (RULE 26)

```
        10         20         30         40         50         60         70         80         90
ACAACACTGCTCTCAGAAGGATACTGCAGAACTCCTTAGAGGTCTTAGCCTATGGAATCATGCTGAAGAGCGACAGAARTTTTTTAAATATTCC

    100        110        120        130        140        150        170        180
GTGGATGAAAAGTCAGATAAAGAAGCAGAAGTGTCAGAACACTCCACAGGTATAACCCATCTTCCTCCTGAGGTAATGCTGTCAATTTTCAGCT

190        200        210        220        230        240        250        260        270        280
ATCTTAATCCTCAAGAGTTATGTCGATGCAGTCAAGTAACCATGAAATGGTCTCAGCTGACAAAAACGGGATCGCCTTTGCAAACATCTTTACCC

    290        300        310        320        330        340        350        360        370
TGTTCATTGGGCCAGAGGTGACTGGTATAGTGGTCCCGCAACTGAACTTGATACTGAACCTGATGATGAATGGGTGAAAAATAGGAAAGATGAA

380        390        400        410        420        430        440        450        460        470
AGTCGTGCTTTTCATGAGTGGGATGAAGATGCTGACATTGATGAATCTGAAGAGTCTGCGGACGAATCAATTGCTATCAGCATTGCACAAATGG

    480        490        500        510        520        530        540        550        560
AAAAACGTTTACTCCATGGCTTAATTCATAACGTTCTACCATATGTTGGTACTTCTGTAAAAACCTTAGTATTAGCATACAGCTCTGCAGTTTC

    570        580        590        600        610        620        630        640        650
CAGCAAAATGGTTAGGCAGATTTTAGAGCTTTGTCCTAACCTGGAGCATCTGGATCTTACCCAGACTGACATTTCAGATTCTGCATTTGACAGT

660        670        680        690        700        710        720        730        740        750
TGGTCTTGGCTTGGTTGCTGCCAGAGTCTTCGGCATCTTGATCTGTCTGGTTGTGAGAAAATCACAGATGTGCCCCTAGAGAAGATTTCCAGAG

    760        770        780        790        800        810        820        830        840
CTCTTGGAATTCTGACATCTCATCAAAGTGGCTTTTTGAAAACATCTACAAGCAAAATTACTTCAACTGCGTGCGAAAAATAAAGACATTACCAT

850        860        870        880        890        900        910        920        930        940
GCAGTCCACCAAGCAGTATGCCTGTTTGCACGATTTAACTAACAAGGGCATTGGAGAAGAAATAGATAATGAACACCCCTGGACTAAGCCTGTT
```

FIG.24B

```
      950       960       970       980       990      1000      1010      1020      1030
TCTTCTGACAATTTCACTTCCTTATGTGTGGATGTTACATGCTGAAGAATTTGGCTGATATTGAAGATACTGTGCAATGGAGACATACAAATG

     1040      1050      1060      1070      1080      1090      1100      1110      1120
TTGAAAGTCTTTGTGTGTAATGGAAACAGCATCCAACTTTAGTTGTTCCACCTCTCGCTTGTTTTACTAAGGACATTGTTGCACTAAGGACTACTGT

     1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
CTGTTGGCAGCAGCATTGTGCTCTGCAGCCCTTTGCCTATTGTGGTCACTCATTTTGTTGTACAGGAACAGCACTTTAAGAACTATGTCATCACTC

     1230      1240      1250      1260      1270      1280      1290      1300      1310
CCAGAATCTTCCTGCAATGTGTAGAAAAGGCAGCAACTACATTGCCTACGCGGAAAACGACTTAATTTACTTTGGGAGTGAAAAATCTGATCAAG

     1320      1330      1340      1350      1360      1370      1380      1390      1400      1410
AGACTGGCAGCTGTACTTCGTGTTTCTCAGTTTATCTGGATGTTATCAGATCACAGACCATGGTCTCAGGGTTTTGACTCTGGGAGCAGGGCTGCC

     1420      1430      1440      1450      1460      1470      1480      1490      1500
TTATTTGGAGCCACCTTAATCTCTCTGCTTGTCTACTATAACTGCTGCAGGCCTGCAGGATTTGGTTTCAGCATGTCCTTCTGTAATGATGAA

     1510      1520      1530      1540      1550      1560      1570      1580      1590
TACTTTTACTACTGTGACAACATTAACGGTCCTCATGCTGATACCGCCAGTGGATGCCAGAATTTGCAGTGTGGTTTTCGAGCCTGCTGCCGCT

     1600      1610      1620      1630      1640      1650      1660      1670      1680      1690
CTGCCCAATGACCCTTGACTTCGATCTTTGTCTACTTCATTTAGCTGACCAGGCTTTCATTGCACTTTACTATCAGCACATTTCTTGTGT

     1700      1710      1720      1730      1740      1750      1760      1770
TAACCATCCCTTTTTGAGCGTGACTTGTTTTGGGCCCATTNYTTACAACTTGAAAATCTTAATTACCAGTGRATTGTAATGTTG
```

FIG.24C

```
        10         20         30         40         50         60
RVTSGCGLARGSSAMVFSNNDEGLINKKLPKELLRIFSFLQIVTLCRCAQISKAWNILA

        70         80         90        100        110        120
LDGSNWQRIDLFNFQIDVEGRVVENISKRCVGFLRKLSLRGCIGVGQSSLKTFAQNCRNI

       130        140        150        160        170        180
EHLNLNGCTKITDSTCYSLSRFCSKLKHLXLTSCVSITNSSLKGISEGCRNLEYLNLSWC

       190        200        210        220        230        240
DQITKDGIEALVRGCRGLKALLLRGCTQLEDEALKHIQNYCHELVSLNLQSCSRITDEGV

       250        260        270        280        290        300
VQICRGCHRLQALCLSGCSNLTDASLTALGLNCPRLQILEAARCSHLTDAGFTLLARNCH

       310        320        330        340        350        360
ELEKMDLEXCILITQSTLIQLSIHCPKLQALSLSHCELIXQDGILHLSNSTCGHERLRVL

       370        380        390        400        410        420
ELDNCLLITQVALXHLENCRGLERLELYDCQQVTRAGIKRMRAQLPHVKVHAYFAPVTPP

       430        440        450        460        470        480
TAVAGSGQRLCRCCVIL*QQLPGPKG**GILSSRRPESS*PTPPSPNLLILHWERHLQFP

       490        500        510        520        530        540
NRHLSRFKNGEDKKGFISNI*HHIVT*NMALT*LVLLLPSSLMSSLTSTHLLL*YL*RLI

       550
ILKTDQTGPASKYINCVQ*
```

FIG.25A

```
         10        20        30        40        50        60        70        80        90
TTTTACTGTACACAGTTGATGTATTTTGATGCTGGGCCTGTCTGGTCTGTCTTGAGGATTATTAACCTTTAGAGGTATCAGAGAAGCAAATGGG

    100       110       120       130       140       150       160       170       180
TACTGGTGAGGCTGCTCATTAGGGAAGAGGGCAAAAGGAGCACTAGCTAGGTCAGAGCCATGTTTCAGGTCACAATGTGATGTCAGATGTTGCT

190       200       210       220       230       240       250       260       270       280
TATAAATCCTTTCTTGTCTTCGCCATTCTTAAATCTTGATAGGTGCCTGTTGGAAACTGTAAATGCCTTTCCCAATGGAGAATCAACAGATTG

      290       300       310       320       330       340       350       360       370
GGTGATGGTGGAGTCGGTCAGGAAGACTCAGGTCTTCTAGAGGAAAGGATGCCTCATCACCCCTTNGGCCCAGGCAGCTGCTGTCAGAGAATGA

 380       390       400       410       420       430       440       450       460       470
CACAGCACCTGCACAGTCGCTGTCCACTTCCTGCCACTGCTGTCGGTGGGGTGACGGGAGCAAAGTAGGCGTGGACTTTGACATGAGGGAGCTG

       480       490       500       510       520       530       540       550       560
AGCCCGCATCCGCTTGATGCCTGCACGGGTAACCTGCTGGCAGTCGTACAGCTCGAGGCGCTCCAGGCCTCGGCAGTTCTCTAGGTGTYCCAGG

   570       580       590       600       610       620       630       640       650
GCCACATCAGTGATGAGGAGGCAGTTGTCCAACTCCAGTACCCGCAGCCTCTCATGGCCACAGGTACTGTTGCTCAGGTGCAGGATCCCATCAT

660       670       680       690       700       710       720       730       740       750
CTGKGATGAGTTCACAGTGGGACAGGCTCAGGGCTTGCAGTTTAGGACAGTGAATGGAGAGCTGGATGAGTGTGCTGTCGGTTATCAGGATGCA

      760       770       780       790       800       810       820       830       840
WTCTTCAAGATCCATCTTCTCCAATTCGTGGCAATTCCGAGCTAAAAGTGTAAAACCTGCGTCAGTCAAATGGGAGCATCGGGCAGCCTCCAAA
```

FIG.25B

SUBSTITUTE SHEET (RULE 26)

```
 850        860        870        880        890        900        910        920        930        940
ATTTGCAGTCGCGGACAGTTCAAACCCAGGGCTGTAAGAGAGGCATCTGTGAGGTTGCTGCAACCCGAAGGCAGACAGCCTGTAGCCGGTGAC

      950        960        970        980        990       1000       1010       1020       1030
AGCCCCTGCATATCTGCACCACACCTTCATCCGTGATACGTGAGCAGGACTGCAAGTTGAGGCTCACAAGCTCATGGCAGTAATTCTGAATGTG

 1040       1050       1060       1070       1080       1090       1100       1110       1120
TTTCAGAGCTTCATCTTCTAACTGTGTGCAGCCCCTCAGGAGCAGGGCTTTCACGCCTCGACAACCTCGCACCAGTGCCTCGATGCCATCCTTC

1130       1140       1150       1160       1170       1180       1190       1200       1210       1220
GTGATCTGATCACACCAAGAGAGGTTCAGGTACTCCAGGTTTCGGCAGCCCTCACTGATCCCCTTCAAGGAGCTGTTTGTAATAGACACACAGG

 1230       1240       1250       1260       1270       1280       1290       1300       1310
ACGTCAGAWCCACATGTTTCAGCTTCGAACAGAATCTGCTAAGGCTATAACACGTGCTGTCAGTGATTTTTGTGCATCCATTGAGGTTCAAATG

1320       1330       1340       1350       1360       1370       1380       1390       1400       1410
TTCAATGTTTCGGCAGTTCTGTGCAAAGGTCTTCAAGGAGGAATCCCCAACACCAATGCAGCCTCGCAAGCTGAGCTTCCTCAGGAATCCAACG

      1420       1430       1440       1450       1460       1470       1480       1490       1500
CATCGCTTCGAGATATTTTCCACCACTCGACCCTCTACATCTATTTGAAAGTTAAAAAGATCTATTCTTTGCCAGTTGCTTCCATCCAGGGCTA

 1510       1520       1530       1540       1550       1560       1570       1580       1590
AGATGTTCCAAGCCTTGCAAATCTGTGCACATCGGCACAAAGTTACTATATCCAAGAAGCAAAATATTCTTAACAGAAGTTCTTTGCGTAACTT

1600       1610       1620       1630       1640       1650       1660       1670       1680
TTTGTTAATAAGGCCTTCATCATTGTTTGAGAAAACCATGGCCGAAGAGCCGCGAGCGAGCCCACAGCCCGAAGTCACACGGC
```

FIG.25C

```
        10         20         30         40         50         60
MSPVFPMLTVLTMFYYICLRRRARTATRGEMMNTHRAIESNSQTSPLNAEVVQYAKEVVD

        70         80         90        100        110        120

FSSHYGSENSMSYTMMNLAGVPNVFPSSGDFTQTAVFRTYGTWMDQCPSASLPFKRTPPN

       130        140        150        160        170        180
FQSQDYVELTFEQCVYPTAVHVLETYHPGAVIRILACSANPYSPNPPAEVRWEILWSERP

       190        200        210        220        230        240
TKVNASQARQFKPCIKQINFPTNLIRLEVNSSLLEYYTELDAVVLHGVKDKPVLSLKTSL

       250        260        270        280        290        300
IDMNDIEDDAYAEKDGCGMDSLNKKFSSAVLGEGPNNGYFDKLPYELIQLILNHLTLPDL

       310        320        330        340        350        360
CRLAQTCKLLSQHCCDPLQYIHLNLQPYWAKLDDTSLEFLQSRCTLVQWLNLSWTGNRGF

       370        380        390        400        410        420
ISVAGFSRFLKVCGSELVRLELSCSHFLNETCLEVISEMCPNLQALNLSSCDKLPPQAFN

       430        440        450        460        470        480
HIAKLCSLKRLVLYRTKVEQTALLSILNFCSELQHLSLGSCVMIEDYDVIASMIGAKCKK

       490        500        510        520        530        540
LRTLDLWRCKNITENGIAELASGCPLLEELDLGWCPTLQSSTGCFTRLAHQLPNLQKLFL

       550        560        570        580        590        600
TANRSVCDTDIDELACNCTRLQQLDILGTRMVSPASLRKLLESCKDLSLLDVSFCSQIDN

       610        620
RAVLELNASFPKVFIKKSFTQ
```

FIG.26A

SUBSTITUTE SHEET (RULE 26)

```
          10         20         30         40         50         60         70         80         90
ATGTCACCGGTCTTTCCCATGTTAACAGTTCTGACCATGTTTTATTATATATGCCTTCGGCCCCGAGCCACGCACAGCTACAAGACGCAGAAATGA

     100        110        120        130        140        150        160        170        180
TGAACACCCATAGAGCTATAGAATCAAACAGCCAGACTTCCCCTCTCAATGCAGAGGTAGTCCAGTATGCCAAAGAAGTAGTGGATTTCAGTTC

190        200        210        220        230        240        250        260        270        280
CCATTATGCGAAGTGAGAATAGTATGTCCTATACTATGTCGAATTTGGCTGGTGTACCAAATGTATTCCCAAGTTCTGGTGACTTTACTCAGACA

      290        300        310        320        330        340        350        360        370
GCTGTGTTTCGAACTTATGGGACATGGTGGCATCAGTGTCCTAGTGCTTCCTTGCCATTCAAGAGGACGCCACCTAATTTTCAGAGCCAGOACT

 380        390        400        410        420        430        440        450        460        470
ATGTGGAACTTACTTTTGAACAACAGGTGTATCCTACAGCTGTACATGTTCTAGAAACCTATCATCCCGGAGCAGTCATTAGAATTCTCGCTTG

      480        490        500        510        520        530        540        550        560
TTCTGCAAATCCTTATTCCCCAAATCCACCAGCTGAAGTAAGATGGGAGATTCTTTGGTCAGAGAGACCTACGAAGGTGAATGCTTCCCAAGCT

  570        580        590        600        610        620        630        640        650
CGCCAGTTTAAACCTTGTATTAAGCAGATAAATTTCCCCACAAATCTTATACGACTGGAAGTAAATAGTTCTCTTCTGGAATATTACACTGAAT

660        670        680        690        700        710        720        730        740        750
TAGATGCAGTTGTGCTACATGGTGTGAAGGACAAGCCAGTGCTTTCTCTCAAGACTTCACTTATTGACATGAATGATATAGAAGATGATGCCTA

      760        770        780        790        800        810        820        830        840
TGCAGAAAAGGATGGTTGTGGAATGGACAGTCTTAACAAAAAGTTTAGCAGTGCTGTCCTCGGGGAAGCGCCAAATAATGGGTATTTTGATAAA

 850        860        870        880        890        900        910        920        930        940
CTACCTTATGAGCTTATTCAGCTGATTCTGAATCATCTTACACTACCAGACCTGTGTAGATTAGCACAGACTTGCAAACTACTGAGCCAGCATT
```

FIG.26B

950 960 970 980 990 1000 1010 1020 1030
GCTGTGATCCTCTGCAATACATCCACCTCAATCTGCAACCATACTGGGCAAAACTAGATGACACTTCTCTGGAATTTCTACAGTCTCGCTGCAC

1040 1050 1060 1070 1080 1090 1100 1110 1120
TCTTGTCCAGTGGCTTAATTTATCTTGGACTGGCAATAGAGGCTTCATCTCTGTTGCAGGATTTACCAGGTTTCTGAAGGTTTGTGGATCCCAA

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220
TTAGTACGCCTTGAATTGTCTTGCAGCCACTTTCTTAATGAAACTTGCTTAGAAGTTATTTCTGAGATGTGTCCAAATCTACAGCCCTTAAATC

1230 1240 1250 1260 1270 1280 1290 1300 1310
TCTCCTCCTGTGATAAGCTACCACCTCAAGCTTTCAACCACATTGCCAAGTTATGCAGCCTTAAACGACTTGTTCTCTATCGAACAAAAGTAGA

1320 1330 1340 1350 1360 1370 1380 1390 1400 1410
GCAAACAGCACTGCTCAGCATTTTGAACTTCTGTTCAGAGCTTCAGCACCTCAGTTTAGGCAGTTGTGTCATGATTGAAGACTATGATGTGATA

1420 1430 1440 1450 1460 1470 1480 1490 1500
GCTAGCATGATAGGAGCCAAGTGTAAAAAACTCCGGACCCTGGATCTGTGGAGATGTAAGAATATTACTGAGAATGGAATAGCAGAACTGGCTT

1510 1520 1530 1540 1550 1560 1570 1580 1590
CTGGGTGTCCACTACTGCAGGAGCTTGACCTTGGCTGGTGCCCAACTCTGCAGAGCAGCACCGGGTGCTTCACCAGACTGGCACACCAGCTCCC

1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
AAACTTGCAAAAACTCTTTCTTACAGCTAATAGATCTGTGTGTGACACAGACATTGATGAATTGGCATGTAATTGTACCAGGTTACAGCAGCTG

1700 1710 1720 1730 1740 1750 1760 1770 1780
GACATATTAGCAACAAGAATGGTAAGTCCGGCATCCTTAAGAAAACTCCTGGAATCTTGTAAAGATCTTTCTTTACTTGATGTGTCCTTCTGTT

1790 1800 1810 1820 1830 1840 1850 1860
CGCAGATTGATAACAGAGCTGTGCTAGAACTGAATGCAAGCTTTCCAAAAGTGTTCATAAAAAAGAGCTTTACTCAGTGA

FIG.26C

```
        10         20         30         40         50         60
MQLVPDIEFKITYTRSPDGDGVGNSYIEDNDDDSKMADLLSYFQQQLTFQESVLKLCQPE

        70         80         90        100        110        120
LESSQIHISVLPMEVLMYIFRMVVSSDLDLRSLEQLSLVCRGFYICARDPEIWRLACLKV

       130        140        150        160        170        180
WGRSCIKLVPYTSWREMFLERPRVRFDGVYISKTTYIRQGEQSLDGFYRAWHQVEYYRYI

       190        200        210        220        230        240
RFFPDCHVMMLTTPEEPQSIVPRLRTRNTRTDAILLGHYRLSQDTDNQTKVFAVITKKKE

       250        260        270        280        290        300
EKPLDYKYRYFRRVPVQEADQSFHVGLQLCSSGHQRFNKLIWIHHSCHITYKSTGETAVS

       310        320
AFEIDKMYTPLFFARVRSYTAFSERPL
```

FIG.27A

```
         10         20         30         40         50         60
ATGCAACTTGTACCTGATATAGAGTTCAAGATTACTTATACCCCGTCTCCAGATGGTGATGGCGTTGGA

70         80         90        100        110        120        130
AACAGCTACATTGAAGATAATGATGATGACAGCAAAATGGCAGATCTCTTGTCCTACTTCCAGCAGCAA

140        150        160        170        180        190        200
CTCACATTTCAGGAGTCTGTGCTTAAACTGTGTCAGCCTGAGCTTGAGAGCAGTCAGATTCACATATCA

210        220        230        240        250        260        270
GTGCTGCCAATGGAGGTCCTGATGTACATCTTCCGATGGGTGGTGTCTAGTGACTTGGACCTCAGATCA

280        290        300        310        320        330        340
TTGGAGCAGTTGTCGCTGGTGTGCAGAGGATTCTACATCTGTGCCAGAGACCCTGAAATATGGCGTCTG

350        360        370        380        390        400        410
GCCTGCTTGAAAGTTTGGGGCAGAAGCTGTATTAAACTTGTTCCGTACACGTCCTGGAGAGAGATGTTT

420        430        440        450        460        470        480
TTAGAACGGCCTCGTGTTCGGTTTGATGGCGTGTATATCAGTAAAACCACATATATTCGTCAAGGGGAA

490        500        510        520        530        540        550
CAGTCTCTTGATGGTTTCTATAGAGCCTGGCACCAAGTGGAATATTACAGGTACATAAGATTCTTTCCT

560        570        580        590        600        610        620
GATGGCCATGTGATGATGTTGACAACCCCTGAAGAGCCTCAGTCCATTGTTCCACGTTTAAGAACTACG

630        640        650        660        670        680        690
AATACCAGGACTGATGCAATTCTACTGGGTCACTATCGCTTGTCACAAGACACAGACAATCAGACCAAA

700        710        720        730        740        750
GTATTTGCTGTAATAACTAAGAAAAAAGAAGAAAAACCACTTGACTATAAATACAGATATTTTCGTCGT

760        770        780        790        800        810        820
GTCCCTGTACAAGAAGCAGATCAGAGTTTTCATGTGGGGCTACAGCTATGTTCCAGTGGTCACCAGAGG

830        840        850        860        870        880        890
TTCAACAAACTCATCTGGATACATCATTCTTGTCACATTACTTACAAATCAACTGGTGAGACTGCAGTC

900        910        920        930        940        950        960
AGTGCTTTGAGATTGACAAGATGTACACCCCCTTGTTCTTCGCCAGAGTAAGGAGCTACACAGCTTTC

970        980
TCAGAAAGGCCTCTGTAG
```

FIG.27B

```
        10         20         30         40         50         60
AALQPDLENDDFFVRKTGAFHANPYVLRAFEDFRKFSEQDDSVERQIILQCREGELVLPD

        70         80         90        100        110        120
LEKDQMIVRRIPAQKKEVPLSGAPQRYHPVPFPEPWTLPPEIQAKFLCVLERTCPSKEKS

       130        140        150        160        170        180
NSCRILVPSYRQKKDQMLTRKIQSWKLGTTVPPISFTPGPCSEADLKRWEAIREASRLRH

       190        200        210        220        230        240
KKRLMVERLFQKIYGENGSKSMSDVSAEDVQNLRQLRYEEMCKIKSQLKEQDQKWQQDLA

       250
KWKQRRKSYTSDLQK
```

FIG.28A

```
         10         20         30         40         50         60
GCAGCCCTGGATCCTGACTTAGAGAATGATGATTTCTTTGTCAGAAAGACTGGGGCTTTCCATGCAAAT

70         80         90        100        110        120        130
CCATATGTTCTCCGAGCTTTTGAAGACTTTAGAAAGTTCTCTGAGCAAGATGATTCTGTAGAGCGAGAT

140        150        160        170        180        190        200
ATAATTTTACAGTGTAGAGAAGGTGAACTTGTACTTCCGGATTTGGAAAAAGATGATATGATTGTTCGC

 210        220        230        240        250        260        270
CGAATCCCAGCACAGAAGAAAGAAGTGCCGCTGTCTGGGGCCCCAGATAGATACCACCCAGTCCCTTTT

 280        290        300        310        320        330        340
CCCGAACCCTGGACTCTTCCTCCAGAAATTCAAGCAAAATTTCTCTGTGTACTTGAAAGGACATGCCCA

  350        360        370        380        390        400        410
TCCAAAGAAAAAAGTAATAGCTGTAGAATATTAGTTCCTTCATATCGGCAGAAGAAAGATGACATGCTG

   420        430        440        450        460        470        480
ACACGTAAGATTCAGTCCTGGAAACTGGGAACTACCGTGCCTCCCATCAGTTTCACNCCTGGCCCCTGC

    490        500        510        520        530        540        550
AGTGAGGCTGACTTGAAGAGATGGGAGCCCATCCGGGAGGCCAGCAGACTCAGGCACAAGAAAAGGCTG

     560        570        580        590        600        610        620
ATGGTGGAGAGACTCTTTCAAAAGATTTATGGTGAGAATGGGAGTAAGTCCATGAGTGATGTCAGCGCA

      630        640        650        660        670        680        690
GAAGATGTTCAAAACTTGCGTCAGCTGCGTTACCAGGAGATGCAGAAAATAAAATCACAATTAAAAGAA

       700        710        720        730        740        750
CAAGATCAGAAATGGCAGGATGACCTTGCAAAATGGAAAGATCGTCGAAAAAGTTACACTTCAGATCTG

760
CAGAAG
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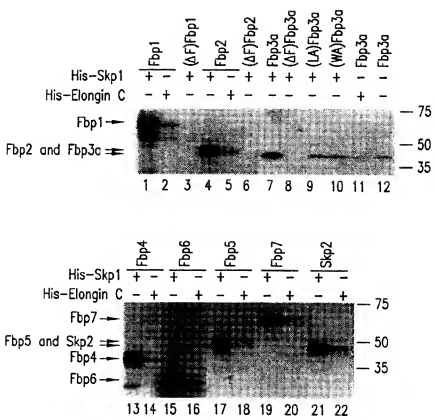
FIG.28B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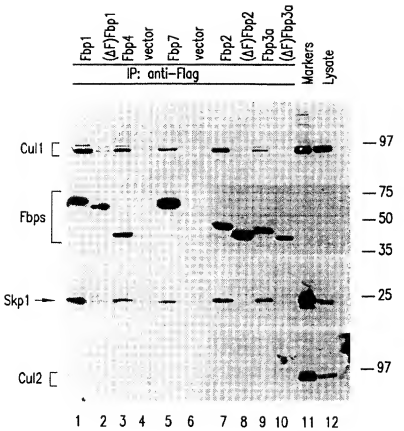
(LA)Fbp3a
(WA)Fbp3a
Fbp3a
Fbp3a
His-Skp1
His-Elongin C
Fbp1
Fbp2 and FbpJc
75
50
35
1 2 3 4 5 6 7 8 9 10 11 12
Fbp4
Fbp6
Fbp5
Fbp7
Skp2
His-Skp1
His-Elongin C
Fbp7
Fbp5 and Skp2
Fbp4
Fbp6
75
50
35
13 14 15 16 17 18 19 20 21 22

FIG.29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Fbp4
vector
Fbp7
vector
Fbp2
(ΔF)Fbp2
Fbp3α
(ΔF)Fbp3α
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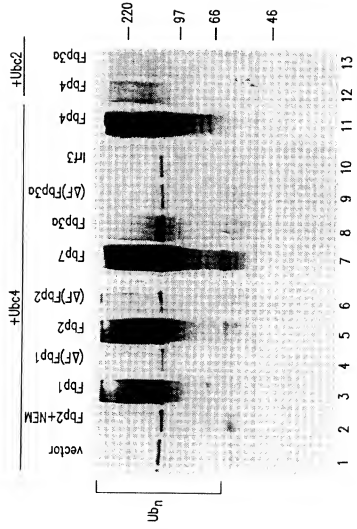
Lysate
IP: anti-Flag
Cul1
Fbps
Skp1
Cul2
97
75
50
35
25
97
1 2 3 4 5 6 7 8 9 10 11 12

FIG.30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Fbp7
(ΔF)Fbp2
Fbp2
(ΔF)Fbp1
Fbp1
Fbp2+NEM
vector
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Ubn

FIG.31

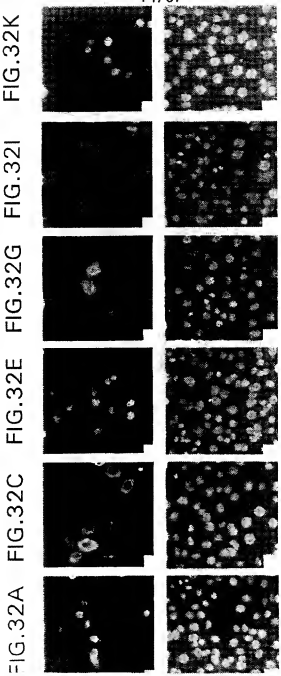
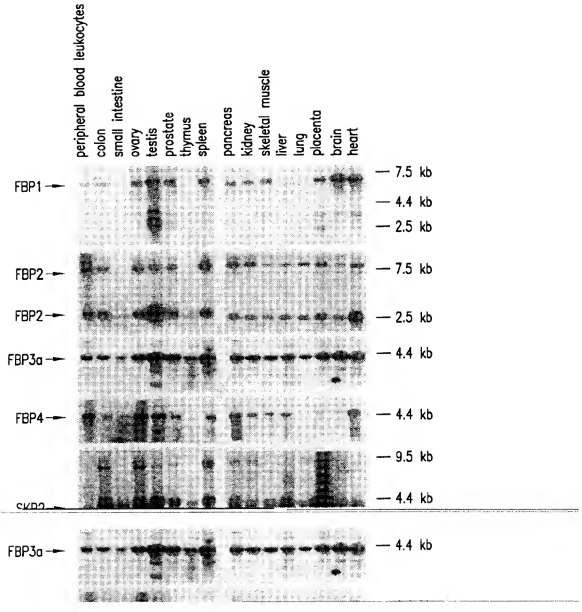


FIG.32A
FIG.32C
FIG.32E
FIG.32G
FIG.32I
FIG.32K
FIG.32B
FIG.32D
FIG.32F
FIG.32H
FIG.32J
FIG.32L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small intestine
ovary
testis
prostate
thymus
spleen
pancreas
kidney
skeletal muscle
liver
lung
placenta
brain
heart
FBP1
FBP2
FBP2
FBP3a
FBP4
SKD3
FBP3a
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4.4 kb

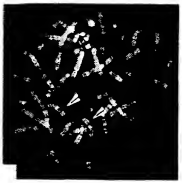


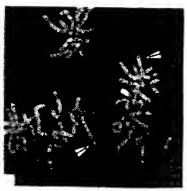
FIG.34A

FIG.34B

FIG.34C

FIG.34D

FIG.34E



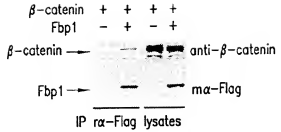


FIG.35A

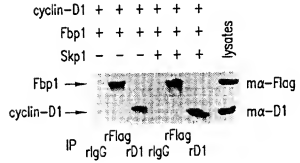


FIG.35B

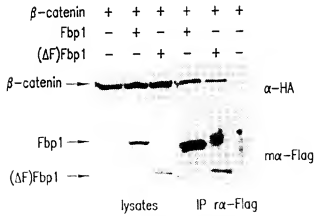


FIG.35C

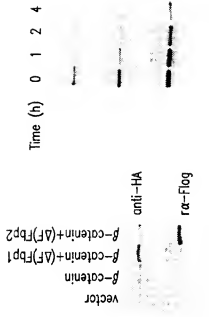


FIG.36A

FIG.36B

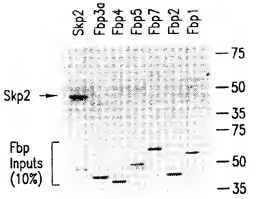


FIG.37A

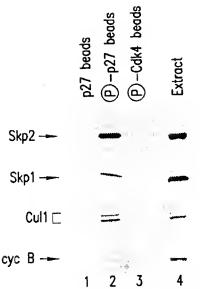


FIG.37B

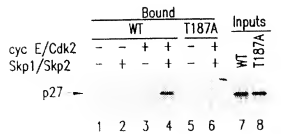
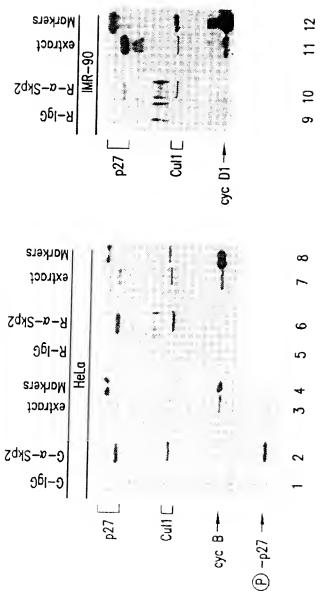


FIG.37C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G–α–Skp2
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Markers
HeLa
R–IgG
R–α–Skp2
extract
Markers
IMR–90
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Cul1
cyc B
Ⓟ –p27
cyc D1
1 2 3 4 5 6 7 8 9 10 11 12

FIG.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19560

A. CLASSIFICATION OF SUBJECT MATTER:
'US CL : '

514/44, 2; 435/455, 69.1, 320.1, 325, 4; 424/93.1, 93.21, 187.1, 800/ 13, 18, 21, 22, 25, 3

Form PCT/ISA/210 (extra sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19560

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database Medline on Dialog, US National Library of Medicine, (Bethesda, MD, USA), No. 95277534, AUFFRAY, C. 'IMAGE: molecular integration of the analysis of the human genome and its expression,' abstract, Comptes Rendus De L Academie Des Sciences. Serie III, Sciences De La Vie, February 1995.	4 --- 5-7

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19360

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 5/00; C12N 15/00, 15/09, 15/63
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44, 2; 435/455, 69.1, 320.1, 325, 4; 424/93.1, 93.21, 187.1; 800/ 13, 18, 21, 22, 25, 3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, MEDLINE, GENBANK, MPSRCH
search terms: ubiquitin ligase, F-box proteins (FBP), knockout, transgenic, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATTON et al. Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. Trends in Genetics. June 1998, Vol. 14, No. 6, pages 236-243, see entire document.	1-27
X, P --- Y, P	NAGASE et al. Prediction of the Coding Sequences of Unidentified Human Genes. XII. The Complete Sequences of 100 New cDNA Clones from Brain Which Code for Large Proteins in vitro. DNA Research. 1998, Vol. 5, pages 355-364, see entire document.	4 --- 5-7
Y	CHISSOE et al. Sequence and Analysis of the Human ABL Gene, the BCR Gene, and Regions Involved in the Philadelphia Chromosomal Translocation. Genomics. 1995, Vol. 27, pages 67-82, see entire document.	4-7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
22 NOVEMBER 1999

Date of mailing of the international search report
23 DEC 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Facsimile No. (703) 305-3230

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JILL D. MARTIN
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Form PCT/ISA/210 (second sheet)(July 1992)*

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<210> 88
<211> 17
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<220>
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catctggcac gatteca 17

<210> 89
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<220>
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Oligonucleotide

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<212> PRT
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<212> PRT
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1 5 10

<210> 85
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Arg Gln Thr

<210> 86
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Asp Arg Ala Cys Ala Ser Ser Val Cys Arg Arg Trp Asn Glu Val Phe
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<213> Homo sapiens

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Arg Arg His Val Gly Ala Gly Leu Cys Trp Ala
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Leu Asp Gly Ser Asn Trp Gln
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<211> 48
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Cys Asp Pro Leu Gln Tyr Ile His Leu Asn Leu Gln Pro Tyr Trp Ala
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<213> Homo sapiens

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Phe Tyr Ile Cys Ala Arg Asp Pro Glu Ile Trp Arg
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<212> PRT
<213> Homo sapiens

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Tyr Arg Gln Lys Lys Asp Asp Met Leu Thr Arg Lys Ile Gln Ser Trp
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Ser Glu Asp Arg Gln Leu Trp Lys
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<213> Homo sapiens

<400> 71
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Gln Leu Cys Arg Cys Ala Arg Val Cys Arg Arg Trp Tyr Asn Leu Ala
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Trp Asp Pro Arg Leu Trp Arg
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<212> PRT
<213> Homo sapiens

<400> 72
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Asp Gly Pro Met Pro Phe Leu Gly Arg Ala Ala Arg Val Cys Arg Arg
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<212> PRT
<213> Homo sapiens

<400> 73
Leu Pro Pro Glu Val Met Leu Ser Ile Phe Ser Tyr Leu Asn Pro Gln
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Lys Thr Gly Ser Leu Trp Lys
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Asp Leu Ile Asn Cys Cys Tyr Val Ser Arg Arg Leu Ser Gln Leu Ser
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Ser His Asp Pro Leu Trp Arg
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<213> Homo sapiens

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Val Asp Gly Ala Pro Leu Trp Leu
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<210> 68
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<213> Homo sapiens

<400> 68
Leu Phe Pro Pro Glu Leu Val Glu His Ile Ile Ser Phe Leu Pro Val
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Cys Asp Gly Glu Gly Val Trp Arg
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Asn Cys Asp Leu Leu Arg Arg Gln Ile Ala Trp Ala
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<212> PRT
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Ala Ala Asp Ile Gly Arg Val Ser Ser Thr Cys Arg Arg Leu Arg Glu
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Leu Cys Gln Ser Ser Gly Lys Val Trp Lys
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<210> 63
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Arg Val Leu Arg Thr His Arg Ser Val Thr Trp Ile
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<213> Homo sapiens

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Leu Ser Leu Asp Ser Thr Arg Trp Arg
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<210> 66
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<213> Homo sapiens

<400> 66

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Ser Gly Ala Pro Asp Arg Tyr His Pro Val Pro Phe Pro Glu Pro Trp
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Thr Leu Pro Pro Glu Ile Gln Ala Lys Phe Leu Cys Val Leu Glu Arg
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Thr Cys Pro Ser Lys Glu Lys Ser Asn Ser Cys Arg Ile Leu Val Pro
        115                 120                 125

Ser Tyr Arg Gln Lys Lys Asp Asp Met Leu Thr Arg Lys Ile Gln Ser
    130                 135                 140

Trp Lys Leu Gly Thr Thr Val Pro Pro Ile Ser Phe Thr Pro Gly Pro
145                 150                 155                 160

Cys Ser Glu Ala Asp Leu Lys Arg Trp Glu Ala Ile Arg Glu Ala Ser
                165                 170                 175

Arg Leu Arg His Lys Lys Arg Leu Met Val Glu Arg Leu Phe Gln Lys
            180                 185                 190

Ile Tyr Gly Glu Asn Gly Ser Lys Ser Met Ser Asp Val Ser Ala Glu
        195                 200                 205

Asp Val Gln Asn Leu Arg Gln Leu Arg Tyr Glu Glu Met Gln Lys Ile
    210                 215                 220

Lys Ser Gln Leu Lys Glu Gln Asp Gln Lys Trp Gln Asp Asp Leu Ala
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Leu Leu Trp Gln
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Gln Glu Ala Asp Gln Ser Phe His Val Gly Leu Gln Leu Cys Ser Ser
            260                 265                 270

Gly His Gln Arg Phe Asn Lys Leu Ile Trp Ile His His Ser Cys His
        275                 280                 285

Ile Thr Tyr Lys Ser Thr Gly Glu Thr Ala Val Ser Ala Phe Glu Ile
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Asp Lys Met Tyr Thr Pro Leu Phe Phe Ala Arg Val Arg Ser Tyr Thr
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Ala Phe Ser Glu Arg Pro Leu
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<222> all n positions
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gattctgtag agcgagatat aattttacag tgtagagaag gtgaacttgt acttccggat 180
ttggaaaaag atgatatgat tgttcgccga atcccagcac agaagaaaga agtgccgctg 240
tctgggccc cagatagata ccacccagtc ccttttcccg aacctggac tcttcctcca 300
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aatagctgta gaatattagt tccttcatat cggcagaaga aagatgacat gctgacacgt 420
aagattcagt cctgaaaact gggaactacc gtgcctccca tcagtttcac ncctggccc 480
tgcagtgagg ctgacttgaa gagatgggag gccatccggg aggccagcag actcaggcac 540
aagaaaaggc tgatggtgga gagactcttt caaaagattt atggtgagaa tgggagtaag 600
tccatgagtg atgtcagcgc agaagatgtt caaaacttgc gtcagctgcg ttacgaggag 660
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aaatggaaag atcgtcgaaa aagttacact tcagatctgc agaag 765

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<211> 255
<212> PRT
<213> Homo sapiens

<400> 60
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            20                  25                  30

Phe Arg Lys Phe Ser Glu Gln Asp Asp Ser Val Glu Arg Asp Ile Ile
        35                  40                  45

Leu Gln Cys Arg Glu Gly Glu Leu Val Leu Pro Asp Leu Glu Lys Asp
    50                  55                  60
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gaaaaaccac ttgactataa atacagatat tttcgtcgtg tcoctgtaca agaagcagat 780
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<213> Homo sapiens

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20 25 30

Asp Ser Lys Met Ala Asp Leu Leu Ser Tyr Phe Gln Gln Gln Leu Thr
35 40 45

Phe Gln Glu Ser Val Leu Lys Leu Cys Gln Pro Glu Leu Glu Ser Ser
50 55 60

Gln Ile His Ile Ser Val Leu Pro Met Glu Val Leu Met Tyr Ile Phe
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Arg Trp Val Val Ser Ser Asp Leu Asp Leu Arg Ser Leu Glu Gln Leu
85 90 95

Ser Leu Val Cys Arg Gly Phe Tyr Ile Cys Ala Arg Asp Pro Glu Ile
100 105 110

Trp Arg Leu Ala Cys Leu Lys Val Trp Gly Arg Ser Cys Ile Lys Leu
115 120 125

Val Pro Tyr Thr Ser Trp Arg Glu Met Phe Leu Glu Arg Pro Arg Val
130 135 140

Arg Phe Asp Gly Val Tyr Ile Ser Lys Thr Thr Tyr Ile Arg Gln Gly
145 150 155 160

Glu Gln Ser Leu Asp Gly Phe Tyr Arg Ala Trp His Gln Val Glu Tyr
165 170 175

Tyr Arg Tyr Ile Arg Phe Phe Pro Asp Gly His Val Met Met Leu Thr
180 185 190

Thr Pro Glu Glu Pro Gln Ser Ile Val Pro Arg Leu Arg Thr Arg Asn
195 200 205

Thr Arg Thr Asp Ala Ile Leu Leu Gly His Tyr Arg Leu Ser Gln Asp
210 215 220

Thr Asp Asn Gln Thr Lys Val Phe Ala Val Ile Thr Lys Lys Lys Glu
225 230 235 240

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Phe Leu Lys Val Cys Gly Ser Glu Leu Val Arg Leu Glu Leu Ser Cys
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Ser His Phe Leu Asn Glu Thr Cys Leu Glu Val Ile Ser Glu Met Cys
385                 390                 395                 400

Pro Asn Leu Gln Ala Leu Asn Leu Ser Ser Cys Asp Lys Leu Pro Pro
                405                 410                 415

Gln Ala Phe Asn His Ile Ala Lys Leu Cys Ser Leu Lys Arg Leu Val
            420                 425                 430

Leu Tyr Arg Thr Lys Val Glu Gln Thr Ala Leu Leu Ser Ile Leu Asn
        435                 440                 445

Phe Cys Ser Glu Leu Gln His Leu Ser Leu Gly Ser Cys Val Met Ile
    450                 455                 460

Glu Asp Tyr Asp Val Ile Ala Ser Met Ile Gly Ala Lys Cys Lys Lys
465                 470                 475                 480

Leu Arg Thr Leu Asp Leu Trp Arg Cys Lys Asn Ile Thr Glu Asn Gly
                485                 490                 495

Ile Ala Glu Leu Ala Ser Gly Cys Pro Leu Leu Glu Glu Leu Asp Leu
            500                 505                 510

Gly Trp Cys Pro Thr Leu Gln Ser Ser Thr Gly Cys Phe Thr Arg Leu
        515                 520                 525

Ala His Gln Leu Pro Asn Leu Gln Lys Leu Phe Leu Thr Ala Asn Arg
    530                 535                 540

Ser Val Cys Asp Thr Asp Ile Asp Glu Leu Ala Cys Asn Cys Thr Arg
545                 550                 555                 560

Leu Gln Gln Leu Asp Ile Leu Gly Thr Arg Met Val Ser Pro Ala Ser
                565                 570                 575

Leu Arg Lys Leu Leu Glu Ser Cys Lys Asp Leu Ser Leu Leu Asp Val
            580                 585                 590

Ser Phe Cys Ser Gln Ile Asp Asn Arg Ala Val Leu Glu Leu Asn Ala
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Ser Phe Pro Lys Val Phe Ile Lys Lys Ser Phe Thr Gln
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Asn Thr His Arg Ala Ile Glu Ser Asn Ser Gln Thr Ser Pro Leu Asn
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Ala Glu Val Val Gln Tyr Ala Lys Glu Val Val Asp Phe Ser Ser His
    50                  55                  60

Tyr Gly Ser Glu Asn Ser Met Ser Tyr Thr Met Trp Asn Leu Ala Gly
65                  70                  75                  80

Val Pro Asn Val Phe Pro Ser Ser Gly Asp Phe Thr Gln Thr Ala Val
                85                  90                  95

Phe Arg Thr Tyr Gly Thr Trp Trp Asp Gln Cys Pro Ser Ala Ser Leu
            100                 105                 110

Pro Phe Lys Arg Thr Pro Pro Asn Phe Gln Ser Gln Asp Tyr Val Glu
        115                 120                 125

Leu Thr Phe Glu Gln Gln Val Tyr Pro Thr Ala Val His Val Leu Glu
    130                 135                 140

Thr Tyr His Pro Gly Ala Val Ile Arg Ile Leu Ala Cys Ser Ala Asn
145                 150                 155                 160

Pro Tyr Ser Pro Asn Pro Pro Ala Glu Val Arg Trp Glu Ile Leu Trp
                165                 170                 175

Ser Glu Arg Pro Thr Lys Val Asn Ala Ser Gln Ala Arg Gln Phe Lys
            180                 185                 190

Pro Cys Ile Lys Gln Ile Asn Phe Pro Thr Asn Leu Ile Arg Leu Glu
        195                 200                 205

Val Asn Ser Ser Leu Leu Glu Tyr Tyr Thr Glu Leu Asp Ala Val Val
    210                 215                 220

Leu His Gly Val Lys Asp Lys Pro Val Leu Ser Leu Lys Thr Ser Leu
225                 230                 235                 240

Ile Asp Met Asn Asp Ile Glu Asp Asp Ala Tyr Ala Glu Lys Asp Gly
                245                 250                 255

Cys Gly Met Asp Ser Leu Asn Lys Lys Phe Ser Ser Ala Val Leu Gly
            260                 265                 270

Glu Gly Pro Asn Asn Gly Tyr Phe Asp Lys Leu Pro Tyr Glu Leu Ile
        275                 280                 285

Gln Leu Ile Leu Asn His Leu Thr Leu Pro Asp Leu Cys Arg Leu Ala
    290                 295                 300

Gln Thr Cys Lys Leu Leu Ser Gln His Cys Cys Asp Pro Leu Gln Tyr
305                 310                 315                 320

Ile His Leu Asn Leu Gln Pro Tyr Trp Ala Lys Leu Asp Asp Thr Ser
                325                 330                 335

Leu Glu Phe Leu Gln Ser Arg Cys Thr Leu Val Gln Trp Leu Asn Leu
            340                 345                 350

Ser Trp Thr Gly Asn Arg Gly Phe Ile Ser Val Ala Gly Phe Ser Arg
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Met Arg Ala Gln Leu Pro His Val Lys Val His Ala Tyr Phe Ala Prc
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Cys Cys Val Ile Leu
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ctggaatctt gtaaagatct ttctttactt gatgtgtcct tctgttcgca gattgataac 1800
agagctgtgc tagaactgaa tgcaagcttt ccaaaagtgt tcataaaaaa gagctttacc 1860
cagtga 1866

<210> 56
<211> 621
<212> PRT
<213> Homo sapiens

<400> 56
Met Ser Pro Val Phe Pro Met Leu Thr Val Leu Thr Met Phe Tyr Tyr
1 5 10 15

Ile Cys Leu Arg Arg Arg Ala Arg Thr Ala Thr Arg Gly Glu Met Met
20 25 30

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Asn Trp Gln Arg Ile Asp Leu Phe Asn Phe Gln Ile Asp Val Glu Gly
 65                  70                  75                  80

Arg Val Val Glu Asn Ile Ser Lys Arg Cys Val Gly Phe Leu Arg Lys
                85                  90                  95

Leu Ser Leu Arg Gly Cys Ile Gly Val Gly Asp Ser Ser Leu Lys Thr
            100                 105                 110

Phe Ala Gln Asn Cys Arg Asn Ile Glu His Leu Asn Leu Asn Gly Cys
        115                 120                 125

Thr Lys Ile Thr Asp Ser Thr Cys Tyr Ser Leu Ser Arg Phe Cys Ser
    130                 135                 140

Lys Leu Lys His Leu Xaa Leu Thr Ser Cys Val Ser Ile Thr Asn Ser
145                 150                 155                 160

Ser Leu Lys Gly Ile Ser Glu Gly Cys Arg Asn Leu Glu Tyr Leu Asn
                165                 170                 175

Leu Ser Trp Cys Asp Gln Ile Thr Lys Asp Gly Ile Glu Ala Leu Val
            180                 185                 190

Arg Gly Cys Arg Gly Leu Lys Ala Leu Leu Leu Arg Gly Cys Thr Gln
        195                 200                 205

Leu Glu Asp Glu Ala Leu Lys His Ile Gln Asn Tyr Cys His Glu Leu
    210                 215                 220

Val Ser Leu Asn Leu Gln Ser Cys Ser Arg Ile Thr Asp Glu Gly Val
225                 230                 235                 240

Val Gln Ile Cys Arg Gly Cys His Arg Leu Gln Ala Leu Cys Leu Ser
                245                 250                 255

Gly Cys Ser Asn Leu Thr Asp Ala Ser Leu Thr Ala Leu Gly Leu Asn
            260                 265                 270

Cys Pro Arg Leu Gln Ile Leu Glu Ala Ala Arg Cys Ser His Leu Thr
        275                 280                 285

Asp Ala Gly Phe Thr Leu Leu Ala Arg Asn Cys His Glu Leu Glu Lys
    290                 295                 300

Met Asp Leu Glu Xaa Cys Ile Leu Ile Thr Asp Ser Thr Leu Ile Gln
305                 310                 315                 320

Leu Ser Ile His Cys Pro Lys Leu Gln Ala Leu Ser Leu Ser His Cys
                325                 330                 335

Glu Leu Ile Xaa Asp Asp Gly Ile Leu His Leu Ser Asn Ser Thr Cys
            340                 345                 350

Gly His Glu Arg Leu Arg Val Leu Glu Leu Asp Asn Cys Leu Leu Ile
        355                 360                 365

Thr Asp Val Ala Leu Xaa His Leu Glu Asn Cys Arg Gly Leu Glu Arg
    370                 375                 380

Leu Glu Leu Tyr Asp Cys Gln Gln Val Thr Arg Ala Gly Ile Lys Arg
385                 390                 395                 400
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<211> 1681
<212> DNA
<213> Homo sapiens

<220>
<221> modified_base
<222> all n positions
<223> n=a, c, g or t

<400> 53
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attaacgttt agaggtatca gagaagcaaa tgggtactgg tgaggctgct cattagggaa 120
gagggcaaaa ggagcactag ctaggtcaga gccatgtttc aggtcaacaat gtgatgtcag 180
atgttgctta taaatccttt cttgtcttcg ccattcttaa atcttgatag gtgcctgttg 240
ggaaactgta aatgcttttc ccaatggaga atcaacagat tgggtgatgg tggagtggat 300
caggaagact caggtcttct agaggaaagg atgcctcatc acccctttngg cccaggcagc 360
tgctgtcaga gaatgacaca gcacctgcac agtcgctgtc cactttcctgc cactgctgtc 420
gttgggtga cggagcaaa gtaggcgtgg actttgacat ggaggagctg agccccgatcc 480
cgcttgatgc ctgcacgggt aacctgctgg cagtcgtaca gctcgcaggcg ctccaggcct 540
cggcagttct ctaggtgtyc cagggccaca tcagtgatga ggaggcagtt gtccaactcc 600
agtaccccgca gcctcttcatg gccacaggta ctgttgctca ggtgcaggat cccatcatct 660
gcgatgaggt cacagtcggga caggctcagg gcttgcaggt taggacagtg aatggagagc 720
tggatgagtg tgctgtcggt tatcaggatg cawtcttcaa gatccatctt ctccaattgg 780
tggcaatttcc gagctaaaag tgtaaaacct gcgtcagtca aatgggagca tcgggcagcc 840
tccaaaattt gcagtcgcgg acagttcaaa cccaggggtg taagagaggc atctgtgagg 900
ttgctgcaac ccgaaaggca gagaggcctgt agccggtgac agccccgtgca tatctgcacc 960
acaccttcat ccgtgatacg tgagcaggac tgcaagtcga ggctcacaag ctcatggcag 1020
taattctgaa tgtgtttcag agcttcatct tctaactgtg tgcagcccct caggagcagg 1080
gctttcaggc ctcgacaacc tcgcaccagt gcctcgatgc catcccttcgt gatctgatca 1140
caccaagaga ggttcaggta ctccaggttt cggcagccct cactgatccc ctcaaggag 1200
ctgtttgtaa tagacacaca ggaggtcaga wccagatgtt tcagcttgga acagaatctg 1260
ctaaggctat aacacgtgct gtcagtgatt tttgtgcatc cattgaggtt caaatgttca 1320
atgttcggc agttctgtgc aaaggtcttc aaggaggaat cccaaacacc aatgcagcct 1380
cgcaagctga gcttcctcag gaatccaacg catcgtcttg agatatttcc cacctctcga 1440
ccctctacat ctatttgaaa gttaaaaaga tctattcttt gccagttgct tccatccagg 1500
gctaagatgt tccaagcctt ggaaatctgt gcacatcggc acaaagttac tatatccaag 1560
aaggaaaata ttcttaacag aagttctttg ggtaacttta tgttaataag gcttcatca 1620
ttgttttgaga aaaccatggc cgaagagccg cgagcgagcc cacagcccga agtcacacgg 1680
c 1681

<210> 54
<211> 437
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> all Xaa positions
<223> Xaa=unknown amino acid residue

<400> 54
Arg Val Thr Ser Gly Cys Gly Leu Ala Arg Gly Ser Ser Ala Met Val
1 5 10 15

Phe Ser Asn Asp Glu Gly Leu Ile Asn Lys Leu Pro Lys Glu
20 25 30

Leu Leu Leu Arg Ile Phe Ser Phe Leu Asp Ile Val Thr Leu Cys Arg
35 40 45

Cys Ala Gln Ile Ser Lys Ala Trp Asn Ile Leu Ala Leu Asp Gly Ser
50 55 60

```
Thr Ala Trp Lys Asn Lys Asp Ile Thr Met Gln Ser Thr Lys Gln Tyr
        275                 280                 285

Ala Cys Leu His Asp Leu Thr Asn Lys Gly Ile Gly Glu Glu Ile Asp
    290                 295                 300

Asn Glu His Pro Trp Thr Lys Pro Val Ser Ser Glu Asn Phe Thr Ser
305                 310                 315                 320

Pro Tyr Val Trp Met Leu Asp Ala Glu Asp Leu Ala Asp Ile Glu Asp
                325                 330                 335

Thr Val Glu Trp Arg His Arg Asn Val Glu Ser Leu Cys Val Met Glu
            340                 345                 350

Thr Ala Ser Asn Phe Ser Cys Ser Thr Ser Gly Cys Phe Ser Lys Asp
        355                 360                 365

Ile Val Gly Leu Arg Thr Ser Val Cys Trp Gln Gln His Cys Ala Ser
    370                 375                 380

Pro Ala Phe Ala Tyr Cys Gly His Ser Phe Cys Cys Thr Gly Thr Ala
385                 390                 395                 400

Leu Arg Thr Met Ser Ser Leu Pro Glu Ser Ser Ala Met Cys Arg Lys
                405                 410                 415

Ala Ala Arg Thr Arg Leu Pro Arg Gly Lys Asp Leu Ile Tyr Phe Gly
            420                 425                 430

Ser Glu Lys Ser Asp Gln Glu Thr Gly Arg Val Leu Leu Phe Leu Ser
        435                 440                 445

Leu Ser Gly Cys Tyr Gln Ile Thr Asp His Gly Leu Arg Val Leu Thr
    450                 455                 460

Leu Gly Gly Gly Leu Pro Tyr Leu Glu His Leu Asn Leu Ser Gly Cys
465                 470                 475                 480

Leu Thr Ile Thr Gly Ala Gly Leu Gln Asp Leu Val Ser Ala Cys Pro
                485                 490                 495

Ser Leu Asn Asp Glu Tyr Phe Tyr Tyr Cys Asp Asn Ile Asn Gly Pro
            500                 505                 510

His Ala Asp Thr Ala Ser Gly Cys Gln Asn Leu Gln Cys Gly Phe Arg
        515                 520                 525

Ala Cys Cys Arg Ser Gly Glu Pro Leu Thr Ser Asp Leu Cys Leu Leu
    530                 535                 540

His Leu Ala Glu Gln Ala Phe Phe His Ala Leu Tyr Ser His Ile Ser
545                 550                 555                 560

Cys Val Asn His Pro Phe Leu Ser Val Thr Cys Phe Gly Pro Ile Xaa
                565                 570                 575

Tyr Asn Phe Arg Asn Leu Asn Tyr Gln Xaa Ile Val Met Leu
            580                 585                 590
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<210> 53

<211> 590
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> all Xaa positions
<223> Xaa=unknown amino acid residue

<400> 52
Gln His Cys Ser Gln Lys Asp Thr Ala Glu Leu Leu Arg Gly Leu Ser
1 5 10 15

Leu Trp Asn His Ala Glu Glu Arg Gln Lys Phe Phe Lys Tyr Ser Val
20 25 30

Asp Glu Lys Ser Asp Lys Glu Ala Glu Val Ser Glu His Ser Thr Gly
35 40 45

Ile Thr His Leu Pro Pro Glu Val Met Leu Ser Ile Phe Ser Tyr Leu
50 55 60

Asn Pro Gln Glu Leu Cys Arg Cys Ser Gln Val Ser Met Lys Trp Ser
65 70 75 80

Gln Leu Thr Lys Thr Gly Ser Leu Trp Lys His Leu Tyr Pro Val His
85 90 95

Trp Ala Arg Gly Asp Trp Tyr Ser Gly Pro Ala Thr Glu Leu Asp Thr
100 105 110

Glu Pro Asp Asp Glu Trp Val Lys Asn Arg Lys Asp Glu Ser Arg Ala
115 120 125

Phe His Glu Trp Asp Glu Asp Ala Asp Ile Asp Glu Ser Glu Glu Ser
130 135 140

Ala Glu Glu Ser Ile Ala Ile Ser Ile Ala Gln Met Glu Lys Arg Leu
145 150 155 160

Leu His Gly Leu Ile His Asn Val Leu Pro Tyr Val Gly Thr Ser Val
165 170 175

Lys Thr Leu Val Leu Ala Tyr Ser Ser Ala Val Ser Ser Lys Met Val
180 185 190

Arg Gln Ile Leu Glu Leu Cys Pro Asn Leu Glu His Leu Asp Leu Thr
195 200 205

Gln Thr Asp Ile Ser Asp Ser Ala Phe Asp Ser Trp Ser Trp Leu Gly
210 215 220

Cys Cys Gln Ser Leu Arg His Leu Asp Leu Ser Gly Cys Glu Lys Ile
225 230 235 240

Thr Asp Val Ala Leu Glu Lys Ile Ser Arg Ala Leu Gly Ile Leu Thr
245 250 255

Ser His Gln Ser Gly Phe Leu Lys Thr Ser Thr Ser Lys Ile Thr Ser
260 265 270

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Ser Ser Gln Thr Thr Ala Ile Leu Gly Ala Leu Leu Gly Ser Cys Cys
        195                 200                 205

Prc Gln Leu Gln Val Leu Glu Val Ser Thr Gly Ile Asn Arg Asn Ser
    210                 215                 220

Ile Pro Leu Gln Leu Pro Val Glu Ala Leu Gln Lys Gly Cys Pro Gln
225                 230                 235                 240

Leu Gln Val Leu Arg Leu Leu Asn Leu Met Trp Leu Pro Lys Pro Pro
                245                 250                 255

Gly Arg Gly Val Ala Pro Gly Pro Gly Phe Pro Ser Leu Glu Leu Leu
            260                 265                 270

Cys Leu Ala Ser Ser Thr Cys Asn Phe Val Ser
        275                 280


<210> 51
<211> 1777
<212> DNA
<213> Homo sapiens

<220>
<221> modified_base
<222> all n positions
<223> n=a, c, g or t

<400> 51
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agaagtgtca gaacactcca caggtatasc ccatcttcct cctgaggtaa tgctgtcaat 180
tttcagctat cttaatcctc aagagttatg tcgatgcagt caagtaagca tgaaatggtc 240
tcagctgaca aaaacgggat cgcttggaa acatctttac cctgttcatt gggccagagg 300
tgactggtat agtggtccog caactgaatc tgatactgaa cctgatgatg aatgggcgaa 360
aaataggaaa gatcaaagtc gtgcttttca tgagtgggat gaagatgctg acattgatga 420
atctaagag tctccggagg aatcaattgc tatcagcatt gcacaaatgg aaaaacgttt 480
actccatggc ttaattcata acgttctacc atatgttggt acttctgtaa aaaccttagt 540
attagcatac agcttgtgcag tttccagcaa aatggtagg cagattttag agcttgtcc 600
taacctggag catctggatc ttacccagac tgacatttca gatctgcat ttgacagttg 660
gtcttggctt ggttgctgcc agagtcttcg gcatcttgat ctgttctggtt gtgagaaaat 720
cacagatgtg gcctagaga agatttccag agtcctgga atttctgacat ctcatcaaag 780
tggctttta aaaacatcta caagcaaact tacttcaact gcgtggaaaa ataaagacat 840
taccatgcag tccaccaagc agtatgcctg tttgcacgat ttaacteaca agggcatgg 900
agaagaaata gataatgaac acccctggac taagcctgtt tcttctgaga atttcacttc 960
tcctatgtg tggatgttag atgctgaaga tttggctgat attgaagata ctgtggaatg 1020
gagatatag aatgttgtaa gtcttgtgt aatggaaaca gcatccaact ttagttgttc 1080
cacctctggt tgttttagta aggacattgt tggactaagg actagtgtct gttggcagca 1140
gcattgtgct tctccagcct ttgcgtattg tggtcactca ttttgttgta caggaacagc 1200
tttangaact atgtcatcac tcccagaatc ttctgcaatg tgtagaaaag cagcaaggac 1260
tagatgcct agggagaaag actaattta ctttgggagt gaaaaatctg atcaagagac 1320
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cagggttttg actctgggag gagggctgcc ttatttggag caccttaatc tctctggttg 1440
tcttactata actggtgcag gcctgcagga tttggtttca gcatgcctt ctctgaatga 1500
tgaatacttt tactactgtg acaacattaa cggtcctcat gctgatacog ccagtggatg 1560
ccagaatttg cagtgtggtt ttcgagcctg ctgccgctct ggcgaatgac ccttgacttc 1620
tgatctttgt ctacttcatt tagctgagca ggctttcttt catgcacttt actcatagca 1680
catttctgt gttaaccatc ccttttgag cgtgactttgt tttgggccca ttnyttacaa 1740
cttcagaaat cttaattacc agtgrattgt aatgttg                        1777

<210> 52
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<211> 850
<212> DNA
<213> Homo sapiens

<400> 49
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ggcggacggc cccatgccct tcctgggcag ggctgcgcgc gtgtgccgcc gctggcagga 180
ggccgcttcc caacccgcgc tctggcacac cgtgacccctg tcgtccccgc tggtcggccg 240
gcctgccaag ggcgggatca aggcggagaa gaagctcctt gcttccctgg agtggcttat 300
gcccaatcgg ttttcacagc tccagaggct gacctctatc cactggaagt ctcaggtaca 360
ccccgtgttg aagctggtag gtgagtgctg tcctcggctc actttcctca agctctccgg 420
ctgccacggt gtgactgctg acgctctggt catgctagcc aaagcctgct gccagctcca 480
tagcctggac ctacagcact ccatgctgga gtccacagct gtggtgagct tcttggagga 540
ggcagggtcc cgaatgcgca agttgtggct gacctacagc tcccagacga cagccatcct 600
gggcgcattg ctgggcagct gctgccccca gctccaggtc ctggaggtga gcaccggcat 660
caaccgtaat agcatttccc ttcagctgcc tgtcgaggct ctgcagaaag gctgccctca 720
gctccagttg ctgcggctgt tgaacctgat gtggctgccc aagcctccgg gacgaggggt 780
ggctcccgga ccaggcttcc ctagcctaga ggagtctgc ctggcgagct caacttgcaa 840
ctttgtgagc 850

<210> 50
<211> 283
<212> PRT
<213> Homo sapiens

<400> 50
Ala Ala Ala Pro Ala Pro Ala Pro Ala Pro Thr Pro Thr Pro Glu Glu
1 5 10 15

Gly Pro Asp Ala Gly Trp Gly Asp Arg Ile Pro Leu Glu Ile Leu Val
20 25 30

Gln Ile Phe Gly Leu Leu Val Ala Ala Asp Gly Pro Met Pro Phe Leu
35 40 45

Gly Arg Ala Ala Arg Val Cys Arg Arg Trp Gln Glu Ala Ala Ser Gln
50 55 60

Pro Ala Leu Trp His Thr Val Thr Leu Ser Ser Pro Leu Val Gly Arg
65 70 75 80

Pro Ala Lys Gly Gly Val Lys Ala Glu Lys Lys Leu Leu Ala Ser Leu
85 90 95

Glu Trp Leu Met Pro Asn Arg Phe Ser Gln Leu Gln Arg Leu Thr Leu
100 105 110

Ile His Trp Lys Ser Gln Val His Pro Val Leu Lys Leu Val Gly Glu
115 120 125

Cys Cys Pro Arg Leu Thr Phe Leu Lys Leu Ser Gly Cys His Gly Val
130 135 140

Thr Ala Asp Ala Leu Val Met Leu Ala Lys Ala Cys Cys Gln Leu His
145 150 155 160

Ser Leu Asp Leu Gln His Ser Met Val Glu Ser Thr Ala Val Val Ser
165 170 175

Phe Leu Glu Glu Ala Gly Ser Arg Met Arg Lys Leu Trp Leu Thr Tyr
180 185 190

Cys Leu Met Leu Glu Thr Val Thr Val Ser Gly Cys Arg Arg Leu Thr
180 185 190

Asp Arg Gly Leu Tyr Thr Ile Ala Gln Cys Cys Pro Glu Leu Arg Arg
195 200 205

Leu Glu Val Ser Gly Cys Tyr Asn Ile Ser Asn Glu Ala Val Phe Asp
210 215 220

Val Val Ser Leu Cys Pro Asn Leu Glu His Leu Asp Val Ser Gly Cys
225 230 235 240

Ser Lys Val Thr Cys Ile Ser Leu Thr Arg Glu Ala Ser Ile Lys Leu
245 250 255

Ser Pro Leu His Gly Lys Gln Ile Ser Ile Arg Tyr Leu Asp Met Thr
260 265 270

Asp Cys Phe Val Leu Glu Asp Glu Gly Leu His Thr Ile Ala Ala His
275 280 285

Cys Thr Gln Leu Thr His Leu Tyr Leu Arg Arg Cys Val Arg Leu Thr
290 295 300

Asp Glu Gly Leu Arg Tyr Leu Val Ile Tyr Cys Ala Ser Ile Lys Glu
305 310 315 320

Leu Ser Val Ser Asp Cys Arg Phe Val Ser Asp Phe Gly Leu Arg Glu
325 330 335

Ile Ala Lys Leu Glu Ser Arg Leu Arg Tyr Leu Ser Ile Ala His Cys
340 345 350

Gly Arg Val Thr Asp Val Gly Ile Arg Tyr Val Ala Lys Tyr Cys Ser
355 360 365

Lys Leu Arg Tyr Leu Asn Ala Arg Gly Cys Glu Gly Ile Thr Asp His
370 375 380

Gly Val Glu Tyr Leu Ala Lys Asn Cys Thr Lys Leu Lys Ser Leu Asp
385 390 395 400

Ile Gly Lys Cys Pro Leu Val Ser Asp Thr Gly Leu Glu Cys Leu Ala
405 410 415

Leu Asn Cys Phe Asn Leu Lys Arg Leu Ser Leu Lys Ser Cys Glu Ser
420 425 430

Ile Thr Gly Gln Gly Leu Gln Ile Val Ala Ala Asn Cys Phe Asp Leu
435 440 445

Gln Thr Leu Asn Val Gln Asp Cys Glu Val Ser Val Glu Ala Leu Arg
450 455 460

Phe Val Lys Arg His Cys Lys Arg Cys Val Ile Glu His Thr Asn Pro
465 470 475 480

Ala Phe Phe

<210> 49

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cctccatgtc tgtctgcgtg ttttccacca aagaatgcaa agcagacttc caggtgttta 2880
aattctgttc actcaacaat gccagatgaa tggaagaggg aacacactga gatgacttag 2940
actctggtcc accaaccaga cccttggaaa ggaatactaa aatcattaca aggtatggat 3000
tttaaatgga tgaaacttca aattatctta tttggataga agtctataatt ctagcctcat 3060
ttgcatgaag tcagatagcc agaagaaatt ccattgctgg ttttcacgaa attcactttgt 3120
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ttacgttgaa atccctcatt tattttcttc tcaaaatgcc cattatccaa atgcagaacc 3240
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ctcctactgt aggctcctgt gcatactgtc gtcttctgtg gtggagtgag aggttagtgt 3360
gatgagttgg tgtctgccca ggaggtttct ttcaaactc atggcctccc atccaatcaa 3420
catcatcaaa ttacatgtgt aatcaaggct ctgtgccatg ggggaaatga atcatttagc 3480
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ttcctcagct ctgtgacttg tgaccctatt tgaagtttca ggatttgggt gtcacaaagg 3600
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gagcagggag atccagagaa tgaatccctg accgcatcac ctaaacttgtc ttccaaacat 3720
gagacaaagc tgactgttca cactgattgc ccagcacata ccgtcttgcc agtttcttct 3780
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gttactggg gaaacagctc agcagatttt tggagaccaa accaaaggtc tcactaggaa 3900
atttatctgt tttaaaacat tgcttccttc ctggctctgc taaattgaat gctcattgtt 3960
tgttgttgtt gtttttaat tctaatgttc aaatcactgc gtgctgtatg natctagaaa 4020
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<210> 48
<211> 483
<212> PRT
<213> Homo sapiens

<400> 48

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Tyr Gly Ser Glu Gly Lys Gly Ser Ser Ser Ile Ser Ser Asp Val Ser
 1               5                  10                  15

Ser Ser Thr Asp His Thr Pro Thr Lys Ala Gln Lys Asn Val Ala Thr
            20                  25                  30

Ser Glu Asp Ser Asp Leu Ser Met Arg Thr Leu Ser Thr Pro Ser Pro
        35                  40                  45

Ala Leu Ile Cys Pro Pro Asn Leu Pro Gly Phe Gln Asn Gly Arg Gly
    50                  55                  60

Ser Ser Thr Ser Ser Ser Ser Ile Thr Gly Glu Thr Val Ala Met Val
65                  70                  75                  80

His Ser Pro Pro Pro Thr Arg Leu Thr His Pro Leu Ile Arg Leu Ala
                85                  90                  95

Ser Arg Pro Gln Lys Glu Gln Ala Ser Ile Asp Arg Leu Pro Asp His
            100                 105                 110

Ser Met Val Gln Ile Phe Ser Phe Leu Pro Thr Asn Gln Leu Cys Arg
        115                 120                 125

Cys Ala Arg Val Cys Arg Arg Trp Tyr Asn Leu Ala Trp Asp Pro Arg
    130                 135                 140

Leu Trp Arg Thr Ile Arg Leu Thr Gly Glu Thr Ile Asn Val Asp Arg
145                 150                 155                 160

Ala Leu Lys Val Leu Thr Arg Arg Leu Cys Gln Asp Thr Pro Asn Val
                165                 170                 175
```

Phe Trp Lys Asp Ser Gly His Pro Cys Thr Ala Ala Asp Pro Asp Ser
245 250 255

Cys Phe Thr Pro Val Ser Pro Gln His Phe Ile Asp Leu Phe Lys Phe
260 265 270

<210> 47
<211> 4059
<212> DNA
<213> Homo sapiens

<400> 47
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tgcgcacact gagcacgccc agcccagccc tgatatgtcc accgaatctc ccaggatttc 180
agaatggaag ggcctcgtcc acctcctcgt cctccatcac cgggaaacg gtggccatgg 240
tgcactcccc gccccgacc cgcctcacac acccgctcat ccggctcgcc tccagcccc 300
agaaggagca ggccagcata gaccggctcc cggaccactc catggtgcag atctttctct 360
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actgggacc gcggctctgg agtactatcc gcctgacggg cgagaccatc aacgtggacc 480
gcgccctcaa ggtgctgacc cgcagactct gccaggacac cccacaagtg tgtctcatgc 540
tggaaaccgt aactgtcagt ggctgcaggc ggctccacaga ccgaggggctg tacaccatcg 600
ccagtgctg ccccgaactg aggcgactgg aagtctcagg ctgttacaat atctccaacg 660
aggccgtctt tgatgtggtg tcctctgtgc ctaatctgga ggacctggat gtgtcaggat 720
gctccaaagt gacctgcatc agcttgaccc gggaggcctc catcaaactg tcacccttgc 780
atggcaaaca gatttccatc cgctacctgg acatgacgga ctgcttcgtg ctggaggacg 840
aaggcctgca caccatcgcg gcgcactgca cgcagctcac ccaccctac ctgcgccgct 900
gcgtccgcct gaccgacgaa ggcctgcgct acctggtgat ctactgcgcc tccatcaagg 960
agctgagcgt cagcgactgc cgcttcgtca gcgacttcgg cctgcgggag atcgccaagc 1020
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gcatccagga ccacggtgtg aggtacctcg ccaggaactg caccaaactc aaatccctgg 1200
atatcggcaa atgcccttttg gtatccgaca cgggcctgga gtgcctggcc cgaactgct 1260
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tgcttagtga agacagacag ctgtggaaga agctttgtca gtaccatttt gctgaaaagc 720
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ttgcgtactc tctc 1214

<210> 46
<211> 272
<212> PRT
<213> Homo sapiens

<400> 46
Leu Ile Leu Tar Ser Val Leu Leu Phe Gln Arg His Gly Tyr Cys Thr
1 5 10 15

Leu Gly Glu Ala Phe Asn Arg Leu Asp Phe Ser Ser Ala Ile Gln Asp
20 25 30

Ile Arg Thr Phe Asn Tyr Val Val Lys Leu Leu Gln Leu Ile Ala Lys
35 40 45

Ser Gln Leu Thr Ser Leu Ser Gly Val Ala Gln Lys Asn Tyr Phe Asn
50 55 60

Ile Leu Asp Lys Ile Val Gln Lys Val Leu Asp Asp His His Asn Pro
65 70 75 80

Arg Leu Ile Lys Asp Leu Leu Gln Asp Leu Ser Ser Thr Leu Cys Ile
85 90 95

Leu Ile Arg Gly Val Gly Lys Ser Val Leu Val Gly Asn Ile Asn Ile
100 105 110

Trp Ile Cys Arg Leu Glu Thr Ile Leu Ala Trp Gln Gln Gln Leu Gln
115 120 125

Asp Leu Gln Met Thr Lys Gln Val Asn Asn Gly Leu Thr Leu Ser Asp
130 135 140

Leu Pro Leu His Met Leu Asn Asn Ile Leu Tyr Arg Phe Ser Asp Gly
145 150 155 160

Trp Asp Ile Ile Thr Leu Gly Gln Val Thr Pro Thr Leu Tyr Met Leu
165 170 175

Ser Glu Asp Arg Gln Leu Trp Lys Lys Leu Cys Gln Tyr His Phe Ala
180 185 190

Glu Lys Gln Phe Cys Arg His Leu Ile Leu Ser Glu Lys Gly His Ile
195 200 205

Glu Trp Lys Leu Met Tyr Phe Ala Leu Gln Lys His Tyr Pro Ala Lys
210 215 220

Glu Gln Tyr Gly Asp Thr Leu His Phe Cys Arg His Cys Ser Ile Leu
225 230 235 240

```
Gly Lys Ile His Ser Thr Phe Ala Ala Lys Tyr Trp Ala His Glu Gln
305                 310                 315                 320

Glu Val Asn Cys Val Asp Cys Lys Gly Gly Ile Ile Ser Phe Gly Ser
                325                 330                 335

Arg Asp Arg Thr Ala Lys Val Trp Pro Leu Ala Ser Gly Gln Leu Gly
            340                 345                 350

Gln Cys Leu Tyr Thr Ile Gln Thr Glu Asp Gln Ile Trp Ser Val Ala
        355                 360                 365

Ile Arg Pro Leu Leu Ser Ser Phe Val Thr Gly Thr Ala Cys Cys Gly
    370                 375                 380

His Phe Ser Pro Leu Lys Ile Trp Asp Leu Asn Ser Gly Gln Leu Met
385                 390                 395                 400

Thr His Leu Asp Arg Asp Phe Pro Pro Arg Ala Gly Val Leu Asp Val
                405                 410                 415

Ile Tyr Glu Ser Pro Phe Ala Leu Leu Ser Cys Gly Tyr Asp Thr Tyr
            420                 425                 430

Val Arg Tyr Trp Asp Cys Arg Thr Ser Val Arg Lys Cys Val Met Glu
        435                 440                 445

Trp Glu Glu Pro His Asn Ser Thr Leu Tyr Cys Leu Gln Thr Asp Gly
    450                 455                 460

Asn His Leu Leu Ala Thr Gly Ser Ser Phe Tyr Ser Val Val Arg Leu
465                 470                 475                 480

Trp Asp Arg His Gln Arg Ala Cys Pro His Thr Phe Pro Leu Thr Ser
                485                 490                 495

Thr Arg Leu Gly Ser Pro Val Tyr Cys Leu His Leu Thr Thr Lys His
            500                 505                 510

Leu Tyr Ala Ala Leu Ser Tyr Asn Leu His Val Leu Asp Ile Gln Asn
        515                 520                 525

Pro
```

<210> 45
<211> 1214
<212> DNA
<213> Homo sapiens

<400> 45
gcattgctat aattttacta tactctcatc taaatctaaa atcagtcttc aaaataaaaa 60
caaattgtcc tttgccaaaa atttttttaa tcgcacaatt aattgacatt aactgccaat 120
tctttttggc taattgacta attttaactt ctgtgttgct tttccagagg catggctatt 180
gcaccttggg agaagcettt aatcggttag acttcttaag tgcaattcaa gatatccgaa 240
cgttcaatta tgtggtcaaa ctgttgcagc taattgcaaa atcccagtta acttcattga 300
gtggcgtggc acagaagaat tacttcaaca ttttggataa aatcgttcaa aaggttcttg 360
atgaccacca caatcctcgc ttaatcaaag atctttctgca agacctaagc tctaccctct 420
gcattcttat tagaggagta gggaagtctg tattagtggg aaacatcaat atttggattt 480
gccgattaga aactattctc gcctggcaac aacagctaca ggatcttcag atgactaagc 540
aagtgaacaa tggcctcacc ctcagtgacc ttcctctgca catgtgtgaac aacatcctat 600

<210> 44
<211> 529
<212> PRT
<213> Homo sapiens

<400> 44
Arg Gly Gly Ser Glu Gly Arg Gly Arg Gly Arg Glu Lys Arg Ala Arg
1 5 10 15

Gly Ala Arg Arg Lys Arg Lys Gln Gly Gly Arg Glu Ala Arg Ala Ala
20 25 30

Asp Gly Glu Gly Gly Ser Gly Pro Gly Ala Glu Ala Gly Ala Arg Thr
35 40 45

Arg Pro Arg Glu Glu Ala Glu Gly Gly Gly Ser Val Glu Glu Gly Ala
50 55 60

Arg Gly Ile Ile Lys Gly Asp Glu Gly Ser Val Gly Ala Gly Lys Glu
65 70 75 80

Ala Gln Gly Arg Lys Tyr Gly Lys Glu Glu Trp Arg Val Arg Ala Arg
85 90 95

Arg Arg Glu Gly Ala Arg Pro Gly Arg Val Gln Gly Gln Gly Gly Gln
100 105 110

Val Trp Ala Tyr Ile Pro Gly Thr Gly Ala Ala Met Ala Ala Ala Ala
115 120 125

Arg Glu Glu Glu Glu Glu Ala Ala Arg Glu Ser Ala Ala Cys Pro Ala
130 135 140

Ala Gly Pro Ala Leu Trp Arg Leu Pro Glu Val Leu Leu Leu His Met
145 150 155 160

Cys Ser Tyr Leu Asp Met Arg Ala Leu Gly Arg Leu Ala Gln Val Tyr
165 170 175

Arg Trp Leu Trp His Phe Thr Asn Cys Asp Leu Leu Arg Arg Gln Ile
180 185 190

Ala Trp Ala Ser Leu Asn Ser Gly Phe Thr Arg Leu Gly Thr Asn Leu
195 200 205

Met Thr Ser Val Pro Val Lys Val Ser Gln Asn Trp Ile Val Gly Cys
210 215 220

Cys Arg Glu Gly Ile Leu Leu Lys Trp Arg Cys Ser Gln Met Pro Trp
225 230 235 240

Met Gln Leu Glu Asp Asp Ala Leu Tyr Ile Ser Gln Ala Asn Phe Ile
245 250 255

Leu Ala Tyr Gln Phe Arg Pro Asp Gly Ala Ser Leu Asn Arg Gln Pro
260 265 270

Leu Gly Val Ser Ala Gly His Asp Glu Asp Val Cys His Phe Val Leu
275 280 285

Ala Thr Ser His Ile Val Ser Ala Gly Gly Asp Gly Lys Ile Gly Leu
290 295 300

```
Pro Arg Cys Asp Thr Val Tyr Arg Lys Tyr Leu Tyr Val Leu Ala Thr
            180                 185                 190

Arg Glu Pro Gln Glu Val Val Gly Thr Thr Ser Ser Arg Ala Cys Asp
        195                 200                 205

Cys Val Glu Val Tyr Leu Gln Ser Ser Gly Gln Arg Val Phe Lys Met
    210                 215                 220

Thr Phe His His Ser Met Thr Phe Lys Gln Ile Val Leu Val Gly Gln
225                 230                 235                 240

Glu Thr Gln Arg Ala Leu Leu Leu Leu Thr Glu Glu Gly Lys Ile Tyr
                245                 250                 255

Ser Leu Val Val Asn Glu Thr Gln Leu Asp Gln Pro Arg Ser Tyr Thr
            260                 265                 270

Val Gln Leu Ala Leu Arg Lys Val Ser His Tyr Leu Pro His Leu Arg
        275                 280                 285

Val Ala Cys Met Thr Ser Asn Gln Ser Ser Thr Leu Tyr Val Thr Asp
    290                 295                 300

Pro Ile Leu Cys Ser Trp Leu Gln Pro Pro Trp Pro Gly Gly
305                 310                 315
```

<210> 43
<211> 1590
<212> DNA
<213> Homo sapiens

<400> 43

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cgaggggga gcgaaggaag gggaagagga agggaaaagc gagcgagagg ggcaaggcgg 60
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ggggctgagg cgggagcgag gacacgccca agaggaggag cagaggggag cggaagcgtg 180
gaggaaggag cgagaggcat catcaaagga catgaggggg agctaggggc cgggaaagag 240
gcacaaggaa gaaagtatgg gaaggaggaa tggagggtca gggctaggcg gcgggaggcc 300
gccaggccgg gaagagtaca aggacaagga ggtcaggattt gggcctacat ccccgggaca 360
ggggcggcca tggcggcggc agccaggaag gaggaggacg aggcggctcg ggagtcagcc 420
gcctgcccgg ctgcgggggcc agcgctctgg cgcctgccgg aagtgctgct gctgacatg 480
tgctcctacc tcgacatgcg ggccctcggc cgcctggccc aggtgtaccg ctggctgtgg 540
cacttcacca actgcgacct gctccggcgc cagatagcct gggcctcgct caactccggc 600
ttcacggcgc tcggaaccaa cctgatgacc agtgtcccag tgaaggtgtc tcagaactgg 660
atagtggggt gctgccgaga ggggattctg ctgaagtggc gatgcagtca gatgccctgg 720
atgcagctag aggatgatgc tttgtacata tccaggctа atttcatcct ggcctaccag 780
ttccgtcgag atggtgccag cttgaaccgt cagcctctgg gagtcctctgc tgggcatgat 840
gaggacgttt gccactttgt gctggccacc tcgcatattg tcagtgcagg aggagatggg 900
aagattggcc ttggtaagat tcacagcacc ttcgctgcca agtactgggc tcatgaacag 960
gaggtgaact gtgtggattg caaagggggc atcatatcat ttggctccag ggacaggacg 1020
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gaagaccaaa tctggtctgt tgctatcagg ccattactca gctcttttgt gacagggacg 1140
gcttgttgtg ggcacttctc acccctgaaa atctgggacc tcaacagtgg gcagctgatg 1200
acacacttgg acagagactt tcccccaagg gctgggggtgc tggatgtcat atatgagtcc 1260
cctttcgcac tgctctcctg tggctatgac acctatgttc gctactgggа ctgccgcacc 1320
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cagacagatg gcaacacctt gcttgccaca ggttcctcct tctatagcgt tgtagggctg 1440
tgggaccggc aocaaagggc ctgccccgcac acctтcccgc tgacgtcgac ccgcctcggc 1500
agcctgtgtt actgcctgca tctcaccacc aagcatctct atgctgcgct gtcttacaac 1560
ctccacgtcc tggtattcca aaaccggtga                                   1590
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<210> 41
<211> 957
<212> DNA
<213> Homo sapiens

<400> 41
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tgtggctcgg agctttgggt tgaagagaag aggggaaag gaaatccgat ttccatccag 120
ttgtctcccc cagagctggt ggagcatatc atctcattcc tccagtcag agacctgtt 180
gcccttggcc agaccgtgcg ctacttccac gaagtgtgcg atgggaagg cgtgtggaga 240
cgcatctgtc gcagactcag tccgcgcctc caagatcagg acacgaaggg cctgtatttc 300
caggcatttg gaggccgccg ccgatgtctc agcaagagcg tggcccccct gctagcccac 360
ggctacgcgc gctcttgtcc caccaaggat cacgtcttca ttcttgacta cgtgggggcc 420
ctcttctttc tcaaaaatgc cctggtctcc accctcggcc agatgcagtg gaagcgggcc 480
tgtcgctatg ttgtgttgtg tcgtggagcc aaggattttg cctcggaccc aaggtgtgac 540
acagtttacc gtaaatacct ctacgtcttg gccactcggg agccgcagga agtggtgggt 600
acccccagca gccgggctgt tgactgtgtt gaggtctatc tgcagtctag tgggcagcgg 660
gtcttcaaga tgacattcca ccactcaatg accttcaagc agatcgtgct ggttggtcag 720
gagacccagc gggctctact gctcctcaca gaggaaggaa agatctactc tttggtagtg 780
aatgagaccc agcttgacca gccacgctcc tacacggttc agctggccct gaggaaggtg 840
tcccactacc tgcctcacct gcgcgtggcc tgcatgactt ccaaccagag cagcaccctc 900
tacgtcacag atcctattct gtgctcttgg ctacaaccac cttcgcctgg tgaatga 957

<210> 42
<211> 318
<212> PRT
<213> Homo sapiens

<400> 42
Met Gly Glu Lys Ala Val Pro Leu Leu Arg Arg Arg Arg Val Lys Arg
1 5 10 15

Ser Cys Pro Ser Cys Gly Ser Glu Leu Gly Val Glu Glu Lys Arg Gly
20 25 30

Lys Gly Asn Pro Ile Ser Ile Gln Leu Phe Pro Pro Glu Leu Val Glu
35 40 45

His Ile Ile Ser Phe Leu Pro Val Arg Asp Leu Val Ala Leu Gly Gln
50 55 60

Thr Cys Arg Cys Tyr Phe His Glu Val Cys Asp Gly Glu Gly Val Trp Arg
65 70 75 80

Arg Ile Cys Arg Arg Leu Ser Pro Arg Leu Gln Asp Gln Asp Thr Lys
85 90 95

Gly Leu Tyr Phe Gln Ala Phe Gly Gly Arg Arg Arg Cys Leu Ser Lys
100 105 110

Ser Val Ala Pro Leu Leu Ala His Gly Tyr Arg Arg Phe Leu Pro Thr
115 120 125

Lys Asp His Val Phe Ile Leu Asp Tyr Val Gly Thr Leu Phe Phe Leu
130 135 140

Lys Asn Ala Leu Val Ser Thr Leu Gly Gln Met Gln Trp Lys Arg Ala
145 150 155 160

Cys Arg Tyr Val Val Leu Cys Arg Gly Ala Lys Asp Phe Ala Ser Asp
165 170 175

gccatcgtgg tgaaggactg gtactcgggc cgcagcgacg ctggttgcct ctacgagctc 540
accgttaagc tactgtccga gcacgagaac gtgctggctg agttcagcag cgggcaggtg 600
gcagtgccca aagacagtga cggcgggggc tggatggaga tctcccacac cttcacccac 660
tacgggcccg gcgtccgctt cgtccgcttc gagcacgggg ggcagggctc cgctactgg 720
aagggctggt tcgggggccg ggtgaccaac agcaggtgt gggtagcacc ctga 774

<210> 40
<211> 257
<212> PRT
<213> Homo sapiens

<400> 40
Ala Ala Ala Ala Ala Ala Tyr Leu Asp Glu Leu Pro Glu Pro Leu Leu
1 5 10 15

Leu Arg Val Leu Ala Ala Leu Pro Ala Ala Glu Leu Val Gln Ala Cys
20 25 30

Arg Leu Val Cys Leu Arg Trp Lys Glu Leu Val Asp Gly Ala Pro Leu
35 40 45

Trp Leu Leu Lys Cys Gln Gln Glu Gly Leu Val Pro Glu Gly Gly Val
50 55 60

Glu Glu Glu Arg Asp His Trp Gln Gln Phe Tyr Phe Leu Ser Lys Arg
65 70 75 80

Arg Arg Asn Leu Leu Arg Asn Pro Cys Gly Glu Glu Asp Leu Glu Gly
85 90 95

Trp Cys Asp Val Glu His Gly Gly Asp Gly Trp Arg Val Glu Glu Leu
100 105 110

Pro Gly Asp Ser Gly Val Glu Phe Thr His Asp Glu Ser Val Lys Lys
115 120 125

Tyr Phe Ala Ser Ser Phe Glu Trp Cys Arg Lys Ala Gln Val Ile Asp
130 135 140

Leu Gln Ala Glu Gly Tyr Trp Glu Glu Leu Leu Asp Thr Thr Gln Pro
145 150 155 160

Ala Ile Val Val Lys Asp Trp Tyr Ser Gly Arg Ser Asp Ala Gly Cys
165 170 175

Leu Tyr Glu Leu Thr Val Lys Leu Leu Ser Glu His Glu Asn Val Leu
180 185 190

Ala Glu Phe Ser Ser Gly Gln Val Ala Val Pro Gln Asp Ser Asp Gly
195 200 205

Gly Gly Trp Met Glu Ile Ser His Thr Phe Thr Asp Tyr Gly Pro Gly
210 215 220

Val Arg Phe Val Arg Phe Glu His Gly Gly Gln Gly Ser Val Tyr Trp
225 230 235 240

Lys Gly Trp Phe Gly Ala Arg Val Thr Asn Ser Ser Val Trp Val Glu
245 250 255

Pro

<222> all n positions
<223> n=a, c, g or t

<400> 37
ggctccggtt tccgggccgg cgggtggccg ctcaccatgc ccggnaagca ccagcatttc 60
caggaacctg aggtcggctg ctgcgggaaa tacttcctgt ttggcttcaa cattgcttcc 120
tgggtgctgg gagcctctgt cctggctatc ggcctctggg cctgggggtga taagggcgtt 180
ctctcgaaca tctcagcgct gacagatctg ggaggccttg accccgtgtg gcttgtttgt 240
ggtagttgga ggcgtcatgt cggtgctggg ctttgctggg ctgcaattgg ggccctccgg 300
gagaacacct tcctgctcaa gtttttctnc gngttcctcg gtctcatctt cttcctggag 360
ctggcaac 368

<210> 38
<211> 122
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> all Xaa positions
<223> Xaa=unknown amino acid residue

<400> 38
Gly Ser Gly Phe Arg Ala Gly Gly Trp Pro Leu Thr Met Pro Gly Lys
1 5 10 15

His Gln His Phe Gln Glu Pro Glu Val Gly Cys Cys Gly Lys Tyr Phe
20 25 30

Leu Phe Gly Phe Asn Ile Val Phe Trp Val Leu Gly Ala Leu Phe Leu
35 40 45

Ala Ile Gly Leu Trp Ala Trp Gly Glu Lys Gly Val Leu Ser Asn Ile
50 55 60

Ser Ala Leu Thr Asp Leu Gly Gly Leu Asp Pro Val Trp Leu Val Cys
65 70 75 80

Gly Ser Trp Arg Arg His Val Gly Ala Gly Leu Cys Trp Ala Ala Ile
85 90 95

Gly Ala Leu Arg Glu Asn Thr Phe Leu Leu Lys Phe Phe Xaa Xaa Phe
100 105 110

Leu Gly Leu Ile Phe Phe Leu Glu Leu Ala
115 120

<210> 39
<211> 774
<212> DNA
<213> Homo sapiens

<400> 39
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gagctcgtgg acggcgcccc gctgtggctg ctcaagtgcc agcaggaggg gctggtgccc 180
gagggcggcg tggaggagga gcgcgaccac tggcagcagt tctacttcct gagcaagcgg 240
cgccgcaacc ttctgcgtaa ccctgtgggg gaagaggact tggaaggctg gtgtgacgtg 300
gagcatggtg gggacggctg gagggtggag gagctgcctg gagacagtgg ggtggagttc 360
acccacgatg agagcgtcaa gaagtacttc gcctcctcct ttgagtggtg tcgcaaagca 420
caggtcattg acctgcaggc tgagggctac tgggaggagc tgctggacac gactcagccg 480

<210> 36
<211> 247
<212> PRT
<213> Homo sapiens

<400> 36
Glu Thr Glu Thr Ala Pro Leu Thr Leu Glu Ser Leu Pro Thr Asp Pro
1 5 10 15

Leu Leu Leu Ile Leu Ser Phe Leu Asp Tyr Arg Asp Leu Ile Asn Cys
20 25 30

Cys Tyr Val Ser Arg Arg Leu Ser Gln Leu Ser Ser His Asp Pro Leu
35 40 45

Trp Arg Arg His Cys Lys Lys Tyr Trp Leu Ile Ser Glu Glu Glu Lys
50 55 60

Thr Gln Lys Asn Gln Cys Trp Lys Ser Leu Phe Ile Asp Thr Tyr Ser
65 70 75 80

Asp Val Gly Arg Tyr Ile Asp His Tyr Ala Ala Ile Lys Lys Ala Ser
85 90 95

Gly Met Ile Ser Arg Asn Ile Trp Ser Pro Gly Val Leu Gly Trp Val
100 105 110

Leu Ser Leu Lys Glu Gly Cys Ser Arg Gly Arg Pro Arg Cys Cys Gly
115 120 125

Ser Ala Asp Trp Ala Ala Ser Phe Leu Asp Asp Tyr Arg Cys Ser Tyr
130 135 140

Arg Ile His Asn Gly Gln Lys Leu Val Gly Ser Trp Gly Tyr Trp Glu
145 150 155 160

Ala Trp His Cys Leu Ile Thr Ile Val Leu Lys Ile Cys Thr Ser Ile
165 170 175

Gln Leu Pro Glu Ile Pro Ala Glu Thr Gly Thr Glu Ile Leu Ser Pro
180 185 190

Phe Asn Phe Cys Ile His Thr Gly Leu Ser Gln Tyr Ile Ala Val Glu
195 200 205

Ala Ala Glu Gly Asn Lys Asn Glu Val Phe Tyr Gln Cys Gln Thr Val
210 215 220

Glu Arg Val Phe Lys Tyr Gly Ile Lys Met Cys Ser Asp Gly Cys Ile
225 230 235 240

Asn Gly Met His Val Phe Ser
245

<210> 37
<211> 368
<212> DNA
<213> Homo sapiens

<220>
<221> modified_base

<210> 34
<211> 179
<212> PRT
<213> Homo sapiens

<400> 34
Arg Pro Arg Pro Gly Leu Arg Gly Gly Arg Ala Pro Cys Glu Val Thr
1 5 10 15

Met Glu Ala Gly Gly Leu Pro Leu Glu Leu Trp Arg Met Ile Leu Ala
20 25 30

Tyr Leu His Leu Pro Asp Leu Gly Arg Cys Ser Leu Val Cys Arg Ala
35 40 45

Trp Tyr Glu Leu Ile Leu Ser Leu Asp Ser Thr Arg Trp Arg Gln Leu
50 55 60

Cys Leu Gly Cys Thr Glu Cys Arg His Pro Asn Trp Pro Asn Gln Pro
65 70 75 80

Asp Val Glu Pro Glu Ser Trp Arg Glu Ala Phe Lys Gln His Tyr Leu
85 90 95

Ala Ser Lys Thr Trp Thr Lys Asn Ala Leu Asp Leu Glu Ser Ser Ile
100 105 110

Cys Phe Ser Leu Phe Arg Arg Arg Arg Glu Arg Arg Thr Leu Ser Val
115 120 125

Gly Pro Gly Arg Glu Phe Asp Ser Leu Gly Ser Ala Leu Ala Met Ala
130 135 140

Ser Leu Tyr Asp Arg Ile Val Leu Phe Pro Gly Val Tyr Glu Glu Gln
145 150 155 160

Gly Glu Ile Ile Leu Lys Val Pro Val Glu Ile Val Gly Gln Gly Lys
165 170 175

Leu Gly

<210> 35
<211> 751
<212> DNA
<213> Homo sapiens

<400> 35
gagaccgaga cggcgccgct gaccctagag tcgctgccca ccgatcccct gctccctatc 60
ttatcctttt tggattatcg ggatctaatc aactgttgtt atgtcagtcg aagattaagc 120
cagctatcaa gtcatgatcc gctgtggaga agacattgca aaaaatactg gctgatatct 180
gaggaagaga aaacacagaa gaatcagtgt tggaaatctc tcttcataga tacttactct 240
gatgtagaaa gatacattga ccattatgct gctatttaaaa aggcctcggg aatgatctca 300
agaaatattt ggagcccagg tgtcctcgga tgggttttat ctctgaaaga ggggtgctcg 360
agaggaagac ctcgatgctg tggaagcgca gattgggctg caagtttcct ggacgattat 420
cgatgttcat accgaattca caatggacag aagttagttg gttcctgggg ttattgggaa 480
gcatggcact gtctaatcac tatcgtctcg aagatttgtt agacgtcgat acagctgccg 540
gagattccag cagagacagg gactgaaata ctgctctccct ttaacttttg catacatact 600
ggtttgagtc agtacatagc agtggaagct gcagaggggtt gaaacaaaaa tgaagttttc 660
taccaatgtc agacagtaga acgtgtgttt aaatatggca ttaagatgtg ttctgatggt 720
tgtataaatg gcatgcatta ggtatttttca g 751

<210> 32
<211> 197
<212> PRT
<213> Homo sapiens

<400> 32
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20 25 30

Pro Pro Gln Gln Gln Gln Gln Gln Gln Pro Pro Pro Pro Pro Pro Pro
35 40 45

Pro Pro Pro Leu Pro Gln Glu Arg Asn Asn Val Gly Glu Arg Asp Asp
50 55 60

Asp Val Pro Ala Asp Met Val Ala Glu Glu Ser Gly Pro Gly Ala Gln
65 70 75 80

Asn Ser Pro Tyr Gln Leu Arg Arg Lys Thr Leu Leu Pro Lys Arg Thr
85 90 95

Ala Cys Pro Thr Lys Asn Ser Met Glu Gly Ala Ser Thr Ser Thr Thr
100 105 110

Glu Asn Phe Gly His Arg Ala Lys Arg Ala Arg Val Ser Gly Lys Ser
115 120 125

Gln Asp Leu Ser Ala Ala Pro Ala Glu Gln Tyr Leu Gln Glu Lys Leu
130 135 140

Pro Asp Glu Val Val Leu Lys Ile Phe Ser Tyr Leu Leu Glu Gln Asp
145 150 155 160

Leu Cys Arg Ala Ala Cys Val Cys Lys Arg Phe Ser Glu Leu Ala Asn
165 170 175

Asp Pro Asn Leu Trp Lys Arg Leu Tyr Met Glu Val Phe Glu Tyr Thr
180 185 190

Arg Pro Met Met His
195

<210> 33
<211> 537
<212> DNA
<213> Homo sapiens

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ccgctgcagc ctggtatgca gggcctggta tgaactgatc ctcagtctcg acagcaccag 180
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<211> 278
<212> DNA
<213> Homo sapiens

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<221> modified_base
<222> all n positions
<223> n=a, c, g or t

<400> 29
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<210> 30
<211> 91
<212> PRT
<213> Homo sapiens

<220>
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<222> all Xaa positions
<223> Xaa=unknown amino acid residue

<400> 30
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Ala Ala Ser Pro Gly Xaa Leu Arg Arg Pro Ala Xaa Thr Phe Val Leu
20 25 30

Ser Asn Leu Ala Glu Val Val Glu Arg Val Leu Thr Phe Leu Pro Ala
35 40 45

Lys Ala Leu Leu Arg Val Ala Cys Val Cys Arg Leu Trp Arg Glu Cys
50 55 60

Val Arg Arg Val Leu Arg Thr His Arg Ser Val Thr Trp Ile Ser Ala
65 70 75 80

Gly Leu Ala Glu Ala Gly His Leu Xaa Gly His
85 90

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<212> DNA
<213> Homo sapiens

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Ser Leu Leu Tyr Leu Thr Ile Ala Arg Gln Leu Gly Val Pro Leu Glu
305                 310                 315                 320

Pro Val Asn Phe Pro Ser His Phe Leu Leu Arg Trp Cys Gln Gly Ala
                325                 330                 335

Glu Gly Ala Thr Leu Asp Ile Phe Asp Tyr Ile Tyr Ile Asp Ala Phe
            340                 345                 350

Gly Lys Gly Lys Gln Leu Thr Val Lys Glu Cys Glu Tyr Leu Ile Gly
        355                 360                 365

Gln His Val Thr Ala Ala Leu Tyr Gly Val Val Asn Val Lys Lys Val
    370                 375                 380

Leu Gln Arg Met Val Gly Asn Leu Leu Ser Leu Gly Lys Arg Glu Gly
385                 390                 395                 400

Ile Asp Gln Ser Tyr Gln Leu Leu Arg Asp Ser Leu Asp Leu Tyr Leu
                405                 410                 415

Ala Met Tyr Pro Asp Gln Val Gln Leu Leu Leu Leu Gln Ala Arg Leu
            420                 425                 430

Tyr Phe His Leu Gly Ile Trp Pro Glu Lys Val Leu Asp Ile Leu Gln
        435                 440                 445

His Ile Gln Thr Leu Asp Pro Gly Gln His Gly Ala Val Gly Tyr Leu
    450                 455                 460

Val Gln His Thr Leu Glu His Ile Glu Arg Lys Lys Glu Glu Val Gly
465                 470                 475                 480

Val Glu Val Lys Leu Arg Ser Asp Glu Lys His Arg Asp Val Cys Tyr
                485                 490                 495

Ser Ile Gly Leu Ile Met Lys His Lys Arg Tyr Gly Tyr Asn Cys Val
            500                 505                 510

Ile Tyr Gly Trp Asp Pro Thr Cys Met Met Gly His Glu Trp Ile Arg
        515                 520                 525

Asn Met Asn Val His Ser Leu Pro His Gly His His Gln Pro Phe Tyr
    530                 535                 540

Asn Val Leu Val Glu Asp Gly Ser Cys Arg Tyr Ala Ala Gln Glu Asn
545                 550                 555                 560

Leu Glu Tyr Asn Val Glu Pro Gln Glu Ile Ser His Pro Asp Val Gly
                565                 570                 575

Arg Tyr Phe Ser Glu Phe Thr Gly Thr His Tyr Ile Pro Asn Ala Glu
            580                 585                 590

Leu Glu Ile Arg Tyr Pro Glu Asp Leu Glu Phe Val Tyr Glu Thr Val
        595                 600                 605

Gln Asn Ile Tyr Ser Ala Lys Lys Glu Asn Ile Asp Glu
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<210> 29

<211> 621
<212> PRT
<213> Homo sapiens

<400> 28
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1 5 10 15

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20 25 30

Leu Pro Gly Glu Val Leu Glu Tyr Ile Leu Cys Cys Gly Ser Leu Thr
35 40 45

Ala Ala Asp Ile Gly Arg Val Ser Ser Thr Cys Arg Arg Leu Arg Glu
50 55 60

Leu Cys Gln Ser Ser Gly Lys Val Trp Lys Glu Gln Phe Arg Val Arg
65 70 75 80

Trp Pro Ser Leu Met Lys His Tyr Ser Pro Thr Asp Tyr Val Asn Trp
85 90 95

Leu Glu Glu Tyr Lys Val Arg Gln Lys Ala Gly Leu Glu Ala Arg Lys
100 105 110

Ile Val Ala Ser Phe Ser Lys Arg Phe Phe Ser Glu His Val Pro Cys
115 120 125

Asn Gly Phe Ser Asp Ile Glu Asn Leu Glu Gly Pro Glu Ile Phe Phe
130 135 140

Glu Asp Glu Leu Val Cys Ile Leu Asn Met Glu Gly Arg Lys Ala Leu
145 150 155 160

Thr Trp Lys Tyr Tyr Ala Lys Lys Ile Leu Tyr Tyr Leu Arg Gln Gln
165 170 175

Lys Ile Leu Asn Asn Leu Lys Ala Phe Leu Gln Gln Pro Asp Asp Tyr
180 185 190

Glu Ser Tyr Leu Glu Gly Ala Val Tyr Ile Asp Gln Tyr Cys Asn Pro
195 200 205

Leu Ser Asp Ile Ser Leu Lys Asp Ile Gln Ala Gln Ile Asp Ser Ile
210 215 220

Val Glu Leu Val Cys Lys Thr Leu Arg Gly Ile Asn Ser Arg His Pro
225 230 235 240

Ser Leu Ala Phe Lys Ala Gly Glu Ser Ser Met Ile Met Glu Ile Glu
245 250 255

Leu Gln Ser Gln Val Leu Asp Ala Met Asn Tyr Val Leu Tyr Asp Gln
260 265 270

Leu Lys Phe Lys Gly Asn Arg Met Asp Tyr Tyr Asn Ala Leu Asn Leu
275 280 285

Tyr Met His Gln Val Leu Ile Arg Arg Thr Gly Ile Pro Ile Ser Met
290 295 300

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Ala Ala Lys Ala Gln Leu Leu Gly Leu Gln Phe Leu Leu Gln Thr Lys
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Ala Thr Gln Gly Leu Ser Arg Tyr Gly Gly Tyr Ile Ser Ala Gly His
            420                 425                 430

Cys Ser Leu Ser Ile Gln Ser Ser Phe Ser Val Gln Pro Phe Phe Leu
        435                 440                 445

Leu Pro Phe Ser Ile Leu Val Ile Ser Leu Gly Asn Ile Ile Leu Gln
    450                 455                 460

Asn Phe Ser Phe Cys Leu Ser Arg Phe Ala Gln Ser Arg Ala Thr Val
465                 470                 475                 480

His Ser Cys Arg Met Ile Asn His Tyr Thr Leu Lys Asp Gly Val Phe
                485                 490                 495

Val His Ile Cys Leu Lys Asn Phe Ile His Phe His Ser Leu Tyr Lys
            500                 505                 510

Tyr His Val Met Cys Thr Tyr Leu Thr Lys Glu Ile Tyr Ser His Asn
        515                 520                 525

Tyr Phe Ile Val Lys Ile Leu Thr Lys Val Phe Pro Phe Leu Ser Asn
    530                 535                 540

Val Leu Lys Phe Ile Phe Ser Glu Thr Ile Val Xaa Val Lys Val Arg
545                 550                 555                 560

Ser Asp Phe Arg Gln Lys Pro Ile Pro Ala Ser Phe Ser Phe Lys Leu
                565                 570                 575

Arg Val Leu Ile Cys Tyr Tyr Ile Thr Met Gln Asn Trp Gln Leu Phe
            580                 585                 590

Leu Tyr Lys Phe Ile Ile Phe Phe Ile Leu Lys Thr Gly Leu Ile Lys
        595                 600                 605

Ser Arg Val Leu Thr Ile Asp Phe Asn Ile Lys Ile Tyr Asp Leu His
    610                 615                 620

Ser Glu Asn Lys Ile Xaa Leu Glu Leu Trp
625                 630
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<210> 27
<211> 4160
<212> DNA
<213> Homo sapiens

<400> 27

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Gly Leu Gln Met Gly Gln Gly Leu Trp Arg Val Val Arg Asn Gln Gln
                85                  90                  95

Leu Gln Gln Glu Gly Tyr Ser Glu Gln Gly Tyr Leu Thr Arg Glu Gln
            100                 105                 110

Ser Arg Arg Met Ala Ala Ser Asn Ile Ser Asn Thr Asn His Arg Lys
        115                 120                 125

Gln Val Gln Gly Gly Ile Asp Ile Tyr His Leu Leu Lys Ala Arg Lys
    130                 135                 140

Ser Lys Glu Gln Glu Gly Phe Ile Asn Leu Glu Met Leu Pro Pro Glu
145                 150                 155                 160

Leu Ser Phe Thr Ile Leu Ser Tyr Leu Asn Ala Thr Asp Leu Cys Leu
                165                 170                 175

Ala Ser Cys Val Trp Gln Asp Leu Ala Asn Asp Glu Leu Leu Trp Gln
            180                 185                 190

Gly Leu Cys Lys Ser Thr Trp Gly His Cys Ser Ile Tyr Asn Lys Asn
        195                 200                 205

Pro Pro Leu Gly Phe Ser Phe Arg Lys Xaa Tyr Met Gln Leu Asp Glu
    210                 215                 220

Gly Ser Leu Thr Phe Asn Ala Asn Pro Asp Glu Gly Val Asn Tyr Phe
225                 230                 235                 240

Met Ser Lys Gly Ile Leu Asp Asp Ser Pro Lys Glu Ile Ala Lys Phe
                245                 250                 255

Ile Phe Cys Thr Arg Thr Leu Asn Trp Lys Lys Leu Arg Ile Tyr Leu
            260                 265                 270

Asp Glu Arg Arg Asp Val Leu Asp Asp Leu Val Thr Leu His Asn Phe
        275                 280                 285

Arg Asn Gln Phe Leu Pro Asn Ala Leu Arg Glu Phe Phe Arg His Ile
    290                 295                 300

His Ala Pro Glu Glu Arg Gly Glu Tyr Leu Glu Thr Leu Ile Thr Lys
305                 310                 315                 320

Phe Ser His Arg Phe Cys Ala Cys Asn Pro Asp Leu Met Arg Glu Leu
                325                 330                 335

Gly Leu Ser Pro Asp Ala Val Tyr Val Leu Cys Tyr Ser Leu Ile Leu
            340                 345                 350

Leu Ser Ile Asp Leu Thr Ser Pro His Val Lys Asn Lys Met Ser Lys
        355                 360                 365

Arg Glu Phe Ile Arg Asn Thr Arg Arg Ala Ala Gln Asn Ile Ser Glu
    370                 375                 380

Asp Phe Val Gly His Leu Tyr Asp Asn Ile Tyr Leu Ile Gly His Val
385                 390                 395                 400
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<211> 1970
<212> DNA
<213> Homo sapiens

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<210> 26
<211> 634
<212> PRT
<213> Homo sapiens

<220>
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<222> all Xaa positions
<223> Xaa=unknown amino acid residue

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Val Thr Ser Thr Gly Val Asp Lys Ser Leu Asn Gln Leu Leu His Gly
35 40 45

Leu Gly Thr Ser Ser Arg Leu Ser His Phe Pro Phe Gly Lys Ser Pro
50 55 60

Ser Ala Glu Ala Ala Cys Asp Ile Leu Ser Gln Leu Val Asn Cys Ser
130 135 140

Ile Gln Thr Leu Gly Leu Ile Ser Thr Ala Lys Pro Ser Phe Met Asn
145 150 155 160

Val Ser Glu Ser His Phe Val Ser Ala Leu Thr Val Val Phe Ile Asn
165 170 175

Ser Lys Ser Leu Ser Ser Ile Lys Ile Glu Asp Thr Pro Val Asp Asp
180 185 190

Pro Ser Leu Lys Ile Leu Val Ala Asn Asn Ser Asp Thr Leu Arg Leu
195 200 205

Pro Lys Met Ser Ser Cys Pro His Val Ser Ser Asp Gly Ile Leu Cys
210 215 220

Val Ala Asp Arg Cys Gln Gly Leu Arg Glu Leu Ala Leu Asn Tyr Tyr
225 230 235 240

Ile Leu Thr Asp Glu Leu Phe Leu Ala Leu Ser Ser Glu Thr His Val
245 250 255

Asn Leu Glu His Leu Arg Ile Asp Val Val Ser Glu Asn Pro Gly Gln
260 265 270

Ile Lys Phe His Ala Val Lys Lys His Ser Trp Asp Ala Leu Ile Lys
275 280 285

His Ser Pro Arg Val Asn Val Val Met His Phe Phe Leu Tyr Glu Glu
290 295 300

Glu Phe Glu Thr Phe Phe Lys Glu Glu Thr Pro Val Thr His Leu Tyr
305 310 315 320

Phe Gly Arg Ser Val Ser Lys Val Val Leu Gly Arg Val Gly Leu Asn
325 330 335

Cys Pro Arg Leu Ile Glu Leu Val Val Cys Ala Asn Asp Leu Gln Pro
340 345 350

Leu Asp Asn Glu Leu Ile Cys Ile Ala Glu His Cys Thr Asn Leu Thr
355 360 365

Ala Leu Gly Leu Ser Lys Cys Glu Val Ser Cys Ser Ala Phe Ile Arg
370 375 380

Phe Val Arg Leu Cys Glu Arg Arg Leu Thr Gln Leu Ser Val Met Glu
385 390 395 400

Glu Val Leu Ile Pro Asp Glu Asp Tyr Ser Leu Asp Glu Ile His Thr
405 410 415

Glu Val Ser Lys Tyr Leu Gly Arg Val Trp Phe Pro Asp Val Met Pro
420 425 430

Leu Trp

<210> 25

<210> 23
<211> 1323
<212> DNA
<213> Homo sapiens

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<213> Homo sapiens

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Gly Ala Ala Lys Gln Pro Lys Val Gly Phe Tyr Ser Ser Leu Asn Gln
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Thr His Thr His Thr Val Leu Leu Asp Trp Gly Ser Leu Pro His His
35 40 45

Val Val Leu Gln Ile Phe Gln Tyr Leu Pro Leu Leu Asp Arg Ala Cys
50 55 60

Ala Ser Ser Val Cys Arg Arg Trp Asn Glu Val Phe His Ile Ser Asp
65 70 75 80

Leu Trp Arg Lys Phe Glu Phe Glu Leu Asn Gln Ser Ala Thr Ser Ser
85 90 95

Phe Lys Ser Thr His Pro Asp Leu Ile Gln Gln Ile Ile Lys Lys His
100 105 110

Phe Ala His Leu Gln Tyr Val Ser Phe Lys Val Asp Ser Ser Ala Glu
115 120 125

<210> 19
<211> 39
<212> PRT
<213> Homo sapiens

<400> 19
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Leu Ile Asn Val Ser Lys Val Ser Thr Thr Trp Lys Lys Ile Leu Glu
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Asp Asp Lys Gly Ala Phe Gln
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<211> 40
<212> PRT
<213> Homo sapiens

<400> 20
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Gln Leu Leu Leu Asn Cys Arg Leu Val Cys Ser Leu Trp Arg Asp Leu
20 25 30

Ile Asp Leu Leu Thr Leu Trp Lys
35 40

<210> 21
<211> 39
<212> PRT
<213> Homo sapiens

<400> 21
Leu Pro Leu Glu Leu Lys Leu Arg Ile Phe Arg Leu Leu Asp Val Arg
1 5 10 15

Ser Val Leu Ser Leu Ser Ala Val Cys Arg Asp Leu Phe Thr Ala Ser
20 25 30

Asn Asp Pro Leu Leu Trp Arg
35

<210> 22
<211> 39
<212> PRT
<213> Homo sapiens

<400> 22
Leu Pro Asp Glu Leu Leu Leu Gly Ile Phe Ser Cys Leu Cys Leu Pro
1 5 10 15

Glu Leu Leu Lys Val Ser Gly Val Cys Lys Arg Trp Tyr Arg Leu Ala
20 25 30

Ser Asp Glu Ser Leu Trp Gln
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<211> 43
<212> PRT
<213> Homo sapiens

<400> 15
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Leu Asp Ala Lys Ser Leu Cys Ala Ala Glu Leu Val Cys Lys Glu Trp
20 25 30

Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys
35 40

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<212> PRT
<213> Homo sapiens

<400> 16
Leu Pro Leu Glu Leu Ser Phe Tyr Leu Leu Lys Trp Leu Asp Pro Gln
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Thr Leu Leu Thr Cys Cys Leu Val Ser Lys Gln Trp Asn Lys Val Ile
20 25 30

Ser Ala Cys Thr Glu Val Trp Gln
35 40

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<212> PRT
<213> Homo sapiens

<400> 17
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Asp Arg Ala His Ala Ser Gln Val Cys Arg Asn Trp Asn Gln Val Phe
20 25 30

His Met Pro Asp Leu Trp Arg
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<210> 18
<211> 39
<212> PRT
<213> Homo sapiens

<400> 18
Leu Pro Ile Asp Val Gln Leu Tyr Ile Leu Ser Phe Leu Ser Pro His
1 5 10 15

Asp Leu Cys Gln Leu Gly Ser Thr Asn His Tyr Trp Asn Glu Thr Val
20 25 30

Arg Asn Pro Ile Leu Trp Arg
35

Thr Glu Ala Lys Ala Leu Ser Leu Pro Glu Lys Trp Lys Leu Ser Gly
180 185 190

Val Tyr Lys Leu Gln Tyr Met His His Leu Cys Glu Gly Ser Ser Ala
195 200 205

Thr Leu Thr Cys Val Pro Leu Gly Asn Leu Ile Val Val Asn Ala Thr
210 215 220

Leu Lys Ile Asn Asn Glu Ile Arg Ser Val Lys Arg Leu Gln Leu Leu
225 230 235 240

Pro Glu Ser Phe Ile Cys Lys Glu Lys Leu Gly Glu Asn Val Ala Asn
245 250 255

Ile Tyr Lys Asp Leu Gln Lys Leu Ser Arg Leu Phe Lys Asp Gln Leu
260 265 270

Val Tyr Pro Leu Leu Ala Phe Thr Arg Gln Ala Leu Asn Leu Pro Asn
275 280 285

Val Phe Gly Leu Val Val Leu Pro Leu Glu Leu Lys Leu Arg Ile Phe
290 295 300

Arg Leu Leu Asp Val Arg Ser Val Leu Ser Leu Ser Ala Val Cys Arg
305 310 315 320

Asp Leu Phe Thr Ala Ser Asn Asp Pro Leu Leu Trp Arg Phe Leu Tyr
325 330 335

Leu Arg Asp Phe Arg Asp Asn Thr Val Arg Val Gln Asp Thr Asp Trp
340 345 350

Lys Glu Leu Tyr Arg Lys Arg His Ile Gln Arg Lys Glu Ser Pro Lys
355 360 365

Gly Arg Phe Val Leu Leu Leu Pro Ser Ser Thr His Thr Ile Pro Phe
370 375 380

Tyr Pro Asn Pro Leu His Pro Arg Pro Phe Pro Ser Ser Arg Leu Pro
385 390 395 400

Pro Gly Ile Ile Gly Gly Glu Tyr Asp Gln Arg Pro Thr Leu Pro Tyr
405 410 415

Val Gly Asp Pro Ile Ser Ser Leu Ile Pro Gly Pro Gly Glu Thr Pro
420 425 430

Ser Gln Leu Pro Pro Leu Arg Pro Arg Phe Asp Pro Val Gly Pro Leu
435 440 445

Pro Gly Pro Asn Pro Ile Leu Pro Gly Arg Gly Gly Pro Asn Asp Arg
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Phe Pro Phe Arg Pro Ser Arg Gly Arg Pro Thr Asp Gly Arg Leu Ser
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Phe Met

<210> 15

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<210> 14
<211> 492
<212> PRT
<213> Homo sapiens

<400> 14
Met Ser Asn Thr Arg Phe Thr Ile Thr Leu Asn Tyr Lys Asp Pro Leu
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Thr Gly Asp Glu Glu Thr Leu Ala Ser Tyr Gly Ile Val Ser Gly Asp
            20                  25                  30

Leu Ile Cys Leu Ile Leu His Asp Asp Ile Pro Pro Pro Asn Ile Pro
        35                  40                  45

Ser Ser Thr Asp Ser Glu His Ser Ser Leu Gln Asn Asn Glu Gln Pro
    50                  55                  60

Ser Leu Ala Thr Ser Ser Asn Gln Thr Ser Ile Gln Asp Glu Gln Pro
65                  70                  75                  80

Ser Asp Ser Phe Gln Gly Gln Ala Ala Gln Ser Gly Val Trp Asn Asp
                85                  90                  95

Asp Ser Met Leu Gly Pro Ser Gln Asn Phe Glu Ala Glu Ser Ile Gln
            100                 105                 110

Asp Asn Ala His Met Ala Glu Gly Thr Gly Phe Tyr Pro Ser Glu Pro
        115                 120                 125

Leu Leu Cys Ser Glu Ser Val Glu Gly Gln Val Pro His Ser Leu Glu
    130                 135                 140

Thr Leu Tyr Gln Ser Ala Asp Cys Ser Asp Ala Asn Asp Ala Leu Ile
145                 150                 155                 160

Val Leu Ile His Leu Leu Met Leu Glu Ser Gly Tyr Ile Pro Gln Gly
                165                 170                 175
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Thr Leu Trp Lys Arg Lys Cys Leu Arg Lys Gly Phe Ile Thr Lys Asp
            100                 105                 110

Trp Asp Gln Pro Val Ala Asp Trp Lys Ile Phe Tyr Phe Leu Arg Ser
        115                 120                 125

Leu His Arg Asn Leu Leu Arg Asn Pro Cys Ala Glu Asn Asp Met Phe
    130                 135                 140

Ala Trp Gln Ile Asp Phe Asn Gly Gly Asp Arg Trp Lys Val Asp Ser
145                 150                 155                 160

Leu Pro Gly Ala His Gly Thr Glu Phe Pro Asp Pro Lys Val Lys Lys
                165                 170                 175

Ser Phe Val Thr Ser Tyr Glu Leu Cys Leu Lys Trp Glu Leu Val Asp
            180                 185                 190

Leu Leu Ala Asp Arg Tyr Trp Glu Glu Leu Leu Asp Thr Phe Arg Pro
        195                 200                 205

Asp Ile Val Val Lys Asp Trp Phe Ala Ala Arg Ala Asp Cys Gly Cys
    210                 215                 220

Thr Tyr Gln Leu Lys Val Gln Leu Ala Ser Ala Asp Tyr Phe Val Leu
225                 230                 235                 240

Ala Ser Phe Glu Pro Pro Pro Val Thr Ile Gln Gln Trp Asn Asn Ala
                245                 250                 255

Thr Trp Thr Glu Val Ser Tyr Thr Phe Ser Asp Tyr Pro Arg Gly Val
            260                 265                 270

Arg Tyr Ile Leu Phe Gln His Gly Gly Arg Asp Thr Gln Tyr Trp Ala
        275                 280                 285

Gly Trp Tyr Gly Pro Arg Val Thr Asn Ser Ser Ile Val Val Ser Pro
    290                 295                 300

Lys Met Thr Arg Asn Gln Ala Ser Ser Glu Ala Gln Pro Gly Gln Lys
305                 310                 315                 320

His Gly Gln Glu Glu Ala Ala Gln Ser Pro Tyr Gly Ala Val Val Gln
                325                 330                 335

Ile Phe
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<210> 13
<211> 1763
<212> DNA
<213> Homo sapiens

<400> 13

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cgcatatggc agagggcaca ggtttctatc cctcagaacc cctgctctgt agtgaatcgg 420
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435 440 445

<210> 11
<211> 1535
<212> DNA
<213> Homo sapiens

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<210> 12
<211> 338
<212> PRT
<213> Homo sapiens

<400> 12
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Val Leu Ser Arg Pro Pro Pro Gly Gly Gly Asp Ser Phe Arg Thr Arg
20 25 30

Arg Pro Gln Arg Gly Pro Gly Pro Gly Gly Ser Gln Ala Met Asp Ala
35 40 45

Pro His Ser Lys Ala Ala Leu Asp Ser Ile Asn Glu Leu Pro Asp Asn
50 55 60

Ile Leu Leu Glu Leu Phe Thr His Val Pro Ala Arg Gln Leu Leu Leu
65 70 75 80

Asn Cys Arg Leu Val Cys Ser Leu Trp Arg Asp Leu Ile Asp Leu Leu
85 90 95

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Gly Ser Pro Ile Val Ser Pro Arg Ile Val Gln Leu Glu Thr Glu Ser
            100                 105                 110

Lys Arg Leu His Asn Lys Glu Asn Gln His Val Gln Gln Thr Leu Asn
        115                 120                 125

Ser Thr Asn Glu Ile Glu Ala Leu Glu Thr Ser Arg Leu Tyr Glu Asp
    130                 135                 140

Ser Gly Tyr Ser Ser Phe Ser Leu Gln Ser Gly Leu Ser Glu His Glu
145                 150                 155                 160

Glu Gly Ser Leu Leu Glu Glu Asn Phe Gly Asp Ser Leu Gln Ser Cys
                165                 170                 175

Leu Leu Gln Ile Gln Ser Pro Asp Gln Tyr Pro Asn Lys Asn Leu Leu
            180                 185                 190

Pro Val Leu His Phe Glu Lys Val Val Cys Ser Thr Leu Lys Lys Asn
        195                 200                 205

Ala Lys Arg Asn Pro Lys Val Asp Arg Glu Met Leu Lys Glu Ile Ile
    210                 215                 220

Ala Arg Gly Asn Phe Arg Leu Gln Asn Ile Ile Gly Arg Lys Met Gly
225                 230                 235                 240

Leu Glu Cys Val Asp Ile Leu Ser Glu Leu Phe Arg Arg Gly Leu Arg
                245                 250                 255

His Val Leu Ala Thr Ile Leu Ala Gln Leu Ser Asp Met Asp Leu Ile
            260                 265                 270

Asn Val Ser Lys Val Ser Thr Thr Trp Lys Lys Ile Leu Glu Asp Asp
        275                 280                 285

Lys Gly Ala Phe Gln Leu Tyr Ser Lys Ala Ile Gln Arg Val Thr Glu
    290                 295                 300

Asn Asn Asn Lys Phe Ser Pro His Ala Ser Thr Arg Glu Tyr Val Met
305                 310                 315                 320

Phe Arg Thr Pro Leu Ala Ser Val Gln Lys Ser Ala Ala Gln Thr Ser
                325                 330                 335

Leu Lys Lys Asp Ala Gln Thr Lys Leu Ser Asn Gln Gly Asp Gln Lys
            340                 345                 350

Gly Ser Thr Tyr Ser Arg His Asn Glu Phe Ser Glu Val Ala Lys Thr
        355                 360                 365

Leu Lys Lys Asn Glu Ser Leu Lys Ala Cys Ile Arg Cys Asn Ser Pro
    370                 375                 380

Ala Lys Tyr Asp Cys Tyr Leu Gln Arg Ala Thr Cys Lys Arg Glu Gly
385                 390                 395                 400

Cys Gly Phe Asp Tyr Cys Thr Lys Cys Leu Cys Asn Tyr His Thr Thr
                405                 410                 415

Lys Asp Cys Ser Asp Gly Lys Leu Leu Lys Ala Ser Cys Lys Ile Gly
            420                 425                 430
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<210> 10
<211> 447
<212> PRT
<213> Homo sapiens

<400> 10
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Cys Ser Ala Ser Pro Ser Ala Val Thr Ala Ala Gly Arg Pro Arg Pro
20 25 30

Ser Asp Ser Cys Lys Glu Glu Ser Ser Thr Leu Ser Val Lys Met Lys
35 40 45

Cys Asp Phe Asn Cys Asn His Val His Ser Gly Leu Lys Leu Val Lys
50 55 60

Pro Asp Asp Ile Gly Arg Leu Val Ser Tyr Thr Pro Ala Tyr Leu Glu
65 70 75 80

Gly Ser Cys Lys Asp Cys Ile Lys Asp Tyr Glu Arg Leu Ser Cys Ile
85 90 95

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Pro Arg Phe Ala Leu Phe Gly Pro Arg Leu Glu Gln Leu Asn Thr Ser
            180                 185                 190

Leu Val Leu Ser Leu Leu Ser Ser Glu Glu Leu Cys Pro Thr Ala Gly
        195                 200                 205

Leu Pro Gln Arg Gln Ile Asp Gly Ile Gly Ser Gly Val Asn Phe Gln
    210                 215                 220

Leu Asn Asn Gln His Lys Phe Asn Ile Leu Ile Leu Tyr Ser Thr Thr
225                 230                 235                 240

Arg Lys Glu Arg Asp Arg Ala Arg Glu Glu His Thr Ser Ala Val Asn
                245                 250                 255

Lys Met Phe Ser Arg His Asn Glu Gly Asp Asp Arg Pro Gly Ser Arg
            260                 265                 270

Tyr Ser Val Ile Pro Gln Ile Gln Lys Leu Cys Glu Val Val Asp Gly
        275                 280                 285

Phe Ile Tyr Val Ala Asn Ala Glu Ala His Lys Arg His Glu Trp Gln
    290                 295                 300

Asp Glu Phe Ser His Ile Met Ala Met Thr Asp Pro Ala Phe Gly Ser
305                 310                 315                 320

Ser Gly Arg Pro Leu Leu Val Leu Ser Cys Ile Ser Gln Gly Asp Val
                325                 330                 335

Lys Arg Met Pro Cys Phe Tyr Leu Ala His Glu Leu His Leu Asn Leu
            340                 345                 350

Leu Asn His Pro Trp Leu Val Gln Asp Thr Glu Ala Glu Thr Leu Thr
        355                 360                 365

Gly Phe Leu Asn Gly Ile Glu Trp Ile Leu Glu Glu Val Glu Ser Lys
    370                 375                 380

Arg Ala Arg Phe Ser Phe Gln Ile Leu Gly Thr Glu Thr Ile Asn Leu
385                 390                 395                 400

Leu Leu Arg Ser Cys Glu Tyr Leu Leu Ser Gln Pro Thr Leu Ser Cys
                405                 410                 415

Leu Phe Ala Asp Arg Leu Ser Phe Gly Gln Leu Leu Leu Cys Phe Leu
            420                 425                 430

Tyr Tyr Phe Tyr Phe Leu Pro Ile Asn Tyr Lys Lys Arg Val Ser Val
        435                 440                 445

Leu Val Phe Ser Pro Lys Met Asn Leu Thr Phe Phe Trp Phe Leu Tyr
    450                 455                 460

Phe Leu Ser Phe Lys Tyr Ile Leu
465                 470
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<210> 9
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<212> DNA
<213> Homo sapiens

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Thr Phe Trp Gln Ser Val Ser Lys Asp Arg Val Ala Arg Thr Thr Ser
        35                  40                  45

Arg Glu Glu Val Asp Glu Ala Ala Ser Thr Leu Thr Arg Leu Pro Ile
    50                  55                  60

Asp Val Gln Leu Tyr Ile Leu Ser Phe Leu Ser Pro His Asp Leu Cys
65                  70                  75                  80

Gln Leu Gly Ser Thr Asn His Tyr Trp Asn Glu Thr Val Arg Asn Pro
                85                  90                  95

Ile Leu Trp Arg Tyr Phe Leu Leu Arg Asp Leu Pro Ser Trp Ser Ser
            100                 105                 110

Val Asp Trp Lys Ser Leu Pro Tyr Leu Gln Ile Leu Lys Lys Pro Ile
        115                 120                 125

Ser Glu Val Ser Asp Gly Ala Phe Phe Asp Tyr Met Ala Val Tyr Leu
    130                 135                 140

Met Cys Cys Pro Tyr Thr Arg Arg Ala Ser Lys Ser Ser Arg Pro Met
145                 150                 155                 160

Tyr Gly Ala Val Thr Ser Phe Leu His Ser Leu Ile Ile Prc Asn Glu
                165                 170                 175
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Ile Ser Ala Leu Thr Val Val Phe Val Asn Ser Lys Ser Leu Ser Ser
                165                 170                 175

Leu Lys Ile Asp Asp Thr Pro Val Asp Asp Pro Ser Leu Lys Val Leu
            180                 185                 190

Val Ala Asn Asn Ser Asp Thr Leu Lys Leu Leu Lys Met Ser Ser Cys
        195                 200                 205

Pro His Val Ser Pro Ala Gly Ile Leu Cys Val Ala Asp Gln Cys His
    210                 215                 220

Gly Leu Arg Glu Leu Ala Leu Asn Tyr His Leu Leu Ser Asp Glu Leu
225                 230                 235                 240

Leu Leu Ala Leu Ser Ser Glu Lys His Val Arg Leu Glu His Leu Arg
                245                 250                 255

Ile Asp Val Val Ser Glu Asn Pro Gly Gln Thr His Phe His Thr Ile
            260                 265                 270

Gln Lys Ser Ser Trp Asp Ala Phe Ile Arg His Ser Pro Lys Val Asn
        275                 280                 285

Leu Val Met Tyr Phe Phe Leu Tyr Glu Glu Glu Phe Asp Pro Phe Phe
    290                 295                 300

Arg Tyr Glu Ile Pro Ala Thr His Leu Tyr Phe Gly Arg Ser Val Ser
305                 310                 315                 320

Lys Asp Val Leu Gly Arg Val Gly Met Thr Cys Pro Arg Leu Val Glu
                325                 330                 335

Leu Val Val Cys Ala Asn Gly Leu Arg Pro Leu Asp Glu Glu Leu Ile
            340                 345                 350

Arg Ile Ala Glu Arg Cys Lys Asn Leu Ser Ala Ile Gly Leu Gly Glu
        355                 360                 365

Cys Glu Val Ser Cys Ser Ala Phe Val Glu Phe Val Lys Met Cys Gly
    370                 375                 380

Gly Arg Leu Ser Gln Leu Ser Ile Met Glu Glu Val Leu Ile Pro Asp
385                 390                 395                 400

Gln Lys Tyr Ser Leu Glu Gln Ile His Trp Glu Val Ser Lys His Leu
                405                 410                 415

Gly Arg Val Trp Phe Pro Asp Met Met Pro Thr Trp
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<212> DNA
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Thr Cys Asp Trp Gly Asn Leu Leu Gln Asp Ile Ile Leu Gln Val Phe
35 40 45

Lys Tyr Leu Pro Leu Leu Asp Arg Ala His Ala Ser Gln Val Cys Arg
50 55 60

Asn Trp Asn Gln Val Phe His Met Pro Asp Leu Trp Arg Cys Phe Glu
65 70 75 80

Phe Glu Leu Asn Gln Pro Ala Thr Ser Tyr Leu Lys Ala Thr His Pro
85 90 95

Glu Leu Ile Lys Gln Ile Ile Lys Arg His Ser Asn His Leu Gln Tyr
100 105 110

Val Ser Phe Lys Val Asp Ser Ser Lys Glu Ser Ala Glu Ala Ala Cys
115 120 125

Asp Ile Leu Ser Gln Leu Val Asn Cys Ser Leu Lys Thr Leu Gly Leu
130 135 140

Ile Ser Thr Ala Arg Pro Ser Phe Met Asp Leu Pro Lys Ser His Phe
145 150 155 160

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Arg Met Lys Gln Leu Glu Asp His Glu Ala Phe Glu Thr Ser Ser Leu
    130                 135                 140

Ile Gly His Ser Ala Arg Val Tyr Ala Leu Tyr Tyr Lys Asp Gly Leu
145                 150                 155                 160

Leu Cys Thr Gly Ser Asp Asp Leu Ser Ala Lys Leu Trp Asp Val Ser
                165                 170                 175

Thr Gly Gln Cys Val Tyr Gly Ile Gln Thr His Thr Cys Ala Ala Val
            180                 185                 190

Lys Phe Asp Glu Gln Lys Leu Val Thr Gly Ser Phe Asp Asn Thr Val
        195                 200                 205

Ala Cys Trp Glu Trp Ser Ser Gly Ala Arg Thr Gln His Phe Arg Gly
    210                 215                 220

His Thr Gly Ala Val Phe Ser Val Asp Tyr Asn Asp Glu Leu Asp Ile
225                 230                 235                 240

Leu Val Ser Gly Ser Ala Asp Phe Thr Val Lys Val Trp Ala Leu Ser
                245                 250                 255

Ala Gly Thr Cys Leu Asn Thr Leu Thr Gly His Thr Glu Trp Val Thr
            260                 265                 270

Lys Val Val Leu Gln Lys Cys Lys Val Lys Ser Leu Leu His Ser Pro
        275                 280                 285

Gly Asp Tyr Ile Leu Leu Ser Ala Asp Lys Tyr Glu Ile Lys Ile Trp
    290                 295                 300

Pro Ile Gly Arg Glu Ile Asn Cys Lys Cys Leu Lys Thr Leu Ser Val
305                 310                 315                 320

Ser Glu Asp Arg Ser Ile Cys Leu Gln Pro Arg Leu His Phe Asp Gly
                325                 330                 335

Lys Tyr Ile Val Cys Ser Ser Ala Leu Gly Leu Tyr Gln Trp Asp Phe
            340                 345                 350

Ala Ser Tyr Asp Ile Leu Arg Val Ile Lys Thr Pro Glu Ile Ala Asn
        355                 360                 365

Leu Ala Leu Leu Gly Phe Gly Asp Ile Phe Ala Leu Leu Phe Asp Asn
    370                 375                 380

Arg Tyr Leu Tyr Ile Met Asp Leu Arg Thr Glu Ser Leu Ile Ser Arg
385                 390                 395                 400

Trp Pro Leu Pro Glu Tyr Arg Glu Ser Lys Arg Gly Ser Ser Phe Leu
                405                 410                 415

Ala Gly Glu His Pro Gly
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<213> Homo sapiens

<211> 1476
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tgcctcgtct ctaaacagcg gaataaggtg atcagtgcct gtacagaggt gtggcagact 300
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aaggtttatt tgaaggctat tttgagaatg aagcaactgg aggaccatga agccttttgaa 420
acctcgtcat taattggaca cagtgccaga gtgtatgcac tttactacaa agatggactt 480
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<212> PRT
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Ile Ser Leu Ser Gly Ala Val Gln Leu Arg His Leu Ser Asn Asn Leu
35 40 45

Glu Thr Leu Leu Lys Arg Asp Phe Leu Lys Leu Leu Pro Leu Glu Leu
50 55 60

Ser Phe Tyr Leu Leu Lys Trp Leu Asp Pro Gln Thr Leu Leu Thr Cys
65 70 75 80

Cys Leu Val Ser Lys Gln Trp Asn Lys Val Ile Ser Ala Cys Thr Glu
85 90 95

Val Trp Gln Thr Ala Cys Lys Asn Leu Gly Trp Gln Ile Asp Asp Ser
100 105 110

Val Gln Asp Ala Leu His Trp Lys Lys Val Tyr Leu Lys Ala Ile Leu
115 120 125

Leu Gln Arg Ile His Cys Arg Ser Glu Thr Ser Lys Gly Val Tyr Cys
260 265 270

Leu Gln Tyr Asp Asp Gln Lys Ile Val Ser Gly Leu Arg Asp Asn Thr
275 280 285

Ile Lys Ile Trp Asp Lys Asn Thr Leu Glu Cys Lys Arg Ile Leu Thr
290 295 300

Gly His Thr Gly Ser Val Leu Cys Leu Gln Tyr Asp Glu Arg Val Ile
305 310 315 320

Ile Thr Gly Ser Ser Asp Ser Thr Val Arg Val Trp Asp Val Asn Thr
325 330 335

Gly Glu Met Leu Asn Thr Leu Ile His His Cys Glu Ala Val Leu His
340 345 350

Leu Arg Phe Asn Asn Gly Met Met Val Thr Cys Ser Lys Asp Arg Ser
355 360 365

Ile Ala Val Trp Asp Met Ala Ser Pro Thr Asp Ile Thr Leu Arg Arg
370 375 380

Val Leu Val Gly His Arg Ala Ala Val Asn Val Val Asp Phe Asp Asp
385 390 395 400

Lys Tyr Ile Val Ser Ala Ser Gly Asp Arg Thr Ile Lys Val Trp Asn
405 410 415

Thr Ser Thr Cys Glu Phe Val Arg Thr Leu Asn Gly His Lys Arg Gly
420 425 430

Ile Ala Cys Leu Gln Tyr Arg Asp Arg Leu Val Val Ser Gly Ser Ser
435 440 445

Asp Asn Thr Ile Arg Leu Trp Asp Ile Glu Cys Gly Ala Cys Leu Arg
450 455 460

Val Leu Glu Gly His Glu Glu Leu Val Arg Cys Ile Arg Phe Asp Asn
465 470 475 480

Lys Arg Ile Val Ser Gly Ala Tyr Asp Gly Lys Ile Lys Val Trp Asp
485 490 495

Leu Val Ala Ala Leu Asp Pro Arg Ala Pro Ala Gly Thr Leu Cys Leu
500 505 510

Arg Thr Leu Val Glu His Ser Gly Arg Val Phe Arg Leu Gln Phe Asp
515 520 525

Glu Phe Gln Ile Val Ser Ser Ser His Asp Asp Thr Ile Leu Ile Trp
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Asp Phe Leu Asn Asp Pro Ala Ala Gln Ala Glu Pro Pro Arg Ser Pro
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Ser Arg Thr Tyr Thr Tyr Ile Ser Arg
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<210> 3

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<212> PRT
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            20                  25                  30

Ile Ile Pro Glu Lys Asn Ser Leu Arg Gln Thr Tyr Asn Ser Cys Ala
        35                  40                  45

Arg Leu Cys Leu Asn Gln Glu Thr Val Cys Leu Ala Ser Thr Ala Met
    50                  55                  60

Lys Thr Glu Asn Cys Val Ala Lys Thr Lys Leu Ala Asn Gly Thr Ser
65                  70                  75                  80

Ser Met Ile Val Pro Lys Gln Arg Lys Leu Ser Ala Ser Tyr Glu Lys
                85                  90                  95

Glu Lys Glu Leu Cys Val Lys Tyr Phe Glu Gln Trp Ser Glu Ser Asp
            100                 105                 110

Gln Val Glu Phe Val Glu His Leu Ile Ser Gln Met Cys His Tyr Gln
        115                 120                 125

His Gly His Ile Asn Ser Tyr Leu Lys Pro Met Leu Gln Arg Asp Phe
    130                 135                 140

Ile Thr Ala Leu Pro Ala Arg Gly Leu Asp His Ile Ala Glu Asn Ile
145                 150                 155                 160

Leu Ser Tyr Leu Asp Ala Lys Ser Leu Cys Ala Ala Glu Leu Val Cys
                165                 170                 175

Lys Glu Trp Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys Lys Leu
            180                 185                 190

Ile Glu Arg Met Val Arg Thr Asp Ser Leu Trp Arg Gly Leu Ala Glu
        195                 200                 205

Arg Arg Gly Trp Gly Gln Tyr Leu Phe Lys Asn Lys Pro Pro Asp Gly
    210                 215                 220

Asn Ala Pro Pro Asn Ser Phe Tyr Arg Ala Leu Tyr Pro Lys Ile Ile
225                 230                 235                 240

Gln Asp Ile Glu Thr Ile Glu Ser Asn Trp Arg Cys Gly Arg His Ser
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SEQUENCE LISTING

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<130> 5914-081-228

<140> To be assigned
<141> 1999-08-27

<150> 60/098,355
<151> 1998-08-28

<150> 60/118,568
<151> 1999-02-03

<150> 60/124,449
<151> 1999-03-15

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<170> PatentIn Ver. 2.0

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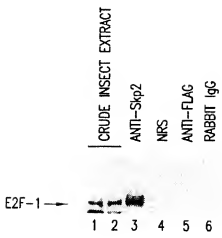
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FIG.44C

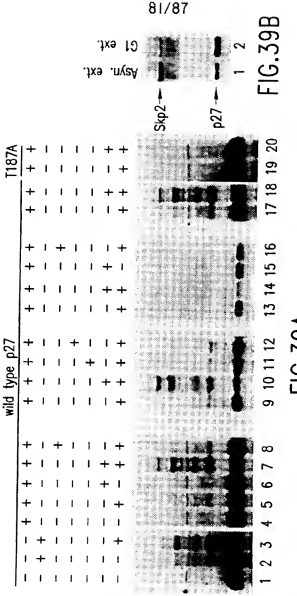
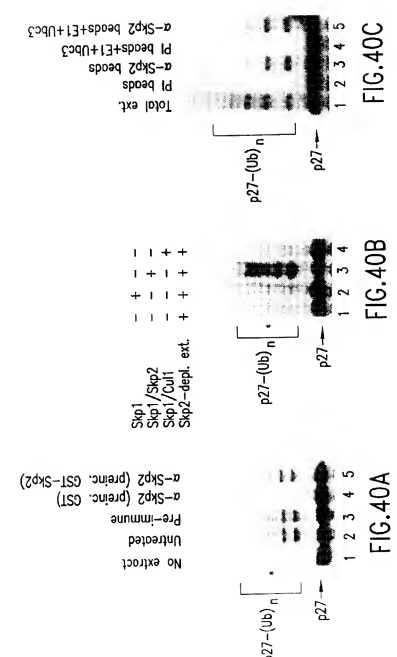


FIG.39A

FIG.39B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Untreated
Pre-immune
α–Skp2 (preinc. GST)
α–Skp2 (preinc. GST–Skp2)
FIG.40A
Skp1
Skp1/Skp2
Skp1/Cul1
Skp2–depl. ext.
– + – + + +
– – + – + +
– – – + + +
+ + + + + +
p27–(Ub)n
p27
1 2 3 4
FIG.40B
Total ext.
PI beads
α–Skp2 beads
PI beads+E1+Ubc3
α–Skp2 beads+E1+Ubc3
p27–(Ub)n
p27
1 2 3 4 5
FIG.40C

FIG.41A

FIG.41B

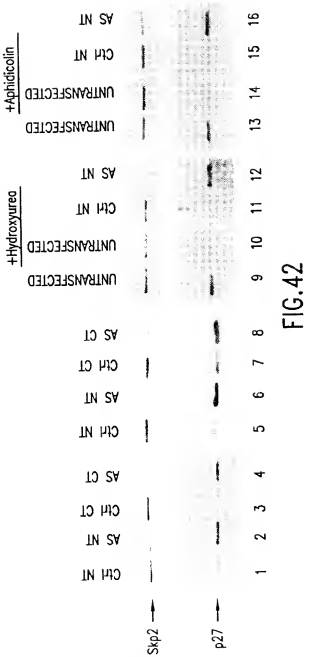
Skp2
p27
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Ctrl NT
AS NT
Ctrl CT
AS CT
Ctrl NT
AS NT
Ctrl CT
AS CT
+Hydroxyurea
UNTRANSFECTED
Ctrl NT
AS NT
+Aphidicolin
UNTRANSFECTED
Ctrl NT
AS NT

FIG.42

SUBSTITUTE SHEET (RULE 26)

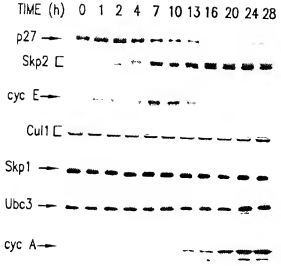

FIG.43A

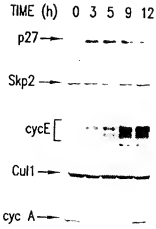

FIG.43B

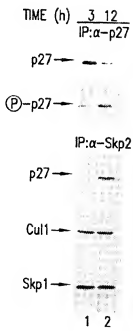

FIG.43C

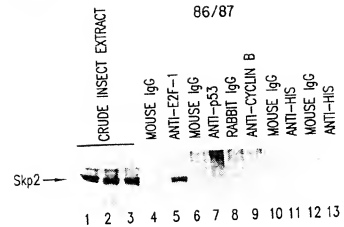

FIG.44A

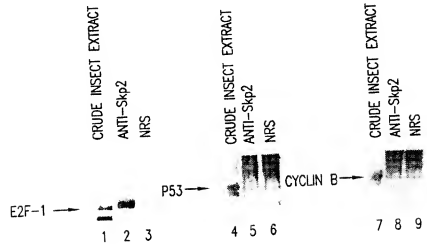

FIG.44B